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STEREOSELECTIVE RELEASE OF SELECTED 2-ARYLPROPIONATES AND FORMULATION-DEPENDENT PHARMACOMETRICS OF TIAPROFENIC ACID

BY

MAJID VAKILYNEJAD ©

A thesis submitted to the Faculty of Graduate Study and Research in partial fulfillment of the requirements for the degree of **DOCTOR of PHILOSOPHY**

IN

PHARMACEUTICAL SCIENCES (PHARMACOKINETICS)

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

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This thesis is dedicated to my parents,

for their unending support and encouragement

ABSTRACT

Stereoselective interaction between enantiomers of a chiral drug with chiral excipient(s) in a pharmaceutical formulation is probable. This study was performed to evaluate possible *in vivo* consequences of *in vitro* stereoselective release of NSAIDs using various formulations of flurbiprofen and tiaprofenic acid (TA). Flurbiprofen release from CM-chitin coated liposomes and sustained release beads containing hydroxypropylmethylcellulose (HPMC)-sodium alginate and HPMC-sodium alginate-dimethyl-β-cyclodextrin (DMCD), at pH 7.4 was not stereoselective. Release of TA enantiomers from sustained release capsules, however, exhibited significant stereoselectivity at pH 7 but this was not evident at pH 8. Similarly, stereoselectivity was observed in the release of TA from the diethyl-β-cyclodextrin (DCD) inclusion complex at pH 3 which was diminished at pH 7.4. There was, however, no difference in the plasma concentration-time profiles of TA enantiomers *in vivo* with respect to the pattern of stereoselectivity in rats following administration of single 20 mg/kg oral dose of TA as powder, inclusion complex and sustained release beads and in humans after 300 mg oral doses of regular release tablets and sustained release capsules.

The modification of NSAID formulations (e.g. enteric coating) to reduce observed gastroduodunal damage may shift the toxicity to the lower intestine. TA-induced upper and lower GI damage after administration of regular release (powder) and modified release formulations (sustained release beads and DCD:TA inclusion complex) were assessed using urinary excretion of GI permeability probes sucrose and ^{51}Cr-EDTA,

respectively. Administration of a regular formulation to rats significantly increased the permeability of gastroduodenal mucosa (a systemic measure of GI damage). Modified release formulations did not damage the proximal GI tract, however, their damage was more pronounced in the lower intestine. To estimate pharmacodynamic parameters of TA a new linked pharmacokinetic-pharmacodynamic model with direct input into the effect compartment was developed.

Modified release formulations of TA exhibited a pH-dependent *in vitro* rate of release and stereoselectivity with no apparent consequence on the pharmacokinetics. However, the possibility of stereoselective release should not be dismissed when evaluating other formulations containing racemic drugs and optically active excipients. Since TA-induced GI damage exhibits formulation dependency, this should be considered in developing new NSAID dosage forms. A parallel evaluation of upper and lower GI toxicity is essential for a complete assessment of NSAID-induced GI damage.

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LIST OF ABBREVIATIONS AND SYMBOLS

μ	Ionic strength
γ	A number influencing the shape of the effect-concentration curve
2-APA	2-Arylpropionic acid
ASA	Acetylsalicylic acid
AUC	Area under the plasma concentration time curve
C	Concentration
C_E	Concentration in the effect compartment
CL/F	Oral clearance
Cl_r	Renal clearance
CL_s	Systemic clearance
C_{max}	Peak concentration
cpm	Counts per minute
DCD	2,6-Diethyl-β-cyclodextrin
DMAP	Diethylaminopyridine
DMCD	Dimethyl-β-cyclodextrin
D	Dose
E	Effect
Emax	Maximum effect

EC_{50}	Concentration of drug corresponding to 50 % of the observed maximum effect
F	Fraction of dose absorbed
FB	Flurbiprofen
GC	Gas chromatography
GI	Gastrointestinal
Hour	h
HPCD	Hydroxypropyl-β-cyclodextrin
HPLC	High performance liquid chromatography
HPMC	Hydroxypropylmethylcellulose
im	Intramuscular
INDP	PK-PD model with direct input into the effect compartment
iv	Intravenous
K_a	First-order absorption rate constant
K_E	Overall first-order elimination rate constant
K_0	Zero-order input into the effect compartment
K_{10}	First-order elimination rate constant from the central compartment
K_{eo}	First-order rate constant for drug loss from the effect compartment
K1e	First-order rate constant for the transfer of drug from the central compartment to the effect compartment

k_P	Partition coefficient at equilibrium
KT	Ketorolac
L	Liter
LUV	Large unilamellar vesicles
M	Molar
min	Minute
mL	Milliter
MLV	Multilamellar vesicles
mM	Millimolar
MRT	Mean transit time
MVV	Multivesicular vesicles
NAP	Naproxen
o/w	Oil-in-water
PD	Pharmacodynamic
PEG 400	Polyethyleneglycol 400
PG	Prostaglandin
PK	Pharmacokinetic
po	Per-oral
RES	Reticulo-endothelial system

REV	Reverse-phase evaporation
S	Second
ΣR24	%Cummulative release within 24 h
SUV	Small unilamellar vesicles
TA	Tiaprofenic acid
TEA	triethylamine
TLC	Thin layer chromatography
t_{max}	The time to peak concentration
TMCD	Trimethyl-β-cyclodextrin
V_d	Volume of distribution
V_E	Volume of the effect compartment
vs	versus
w/o	Water-in-oil

1. INTRODUCTION

The building blocks of a living organism consist of carbon-based macromolecules which possess a central asymmetry. Four different moieties can be attached to the carbon atom creating a 3-dimensional spatial structure (*tetrahedron)*. This spatial arrangement of atoms around a centrally located carbon atom is crucial for the preferential and selective interaction of xenobiotics with biomolecules. It seems that the biological milieus favor one spatial intermolecular order over other possible combinations. Hence qualitative and quantitative differences in pharmacokinetics (PK) and pharmacodynamics (PD) of enantiomers of drugs with one or more asymmetric carbon atoms are expected.

In recent years, pharmaceutical scientists have devoted considerable attention to the issue of drug chirality (Borman,1990 and Jamali *et al.*,1989). Although substantial research has been done on the PK, PD, toxicological, and regulatory aspects of chirality (Drayer, 1986; Ariens, 1984; Jamali *et al.*,1989, Ariens, 1991; Jamali, 1994), the importance of stereochemistry in pharmaceutics and physical pharmacy has not been adequately addressed. Chirality and enantiomeric purity of compounds can greatly influence salt formation and properties of optically-active crystals (Duddu *et al.*, 1993). While a large number of chiral excipients (Table 1.1), such as cellulose polymers and cyclodextrins, are used in modern pharmaceutical formulations (Grass *et al.*, 1990), the possibility of preferential interaction of enantiomers of a racemate with these additives remains to be investigated.

Table 1.1. Chiral excipients commonly used in the pharmaceutical industry

Excipients	Pharmaceutical application
Alginic acid	Disintegrant, tablet binder
Ascorbic acid	Antioxidant
Carboxymethylcellulose calcium	Disintegrant
Carboxymethylcellulose sodium	Disintegrant
Cyclodextrin and its derivatives	Complexing agent, dissolution enhancer, complexing agent in preparation of sustained release granules
Dextrin	Adhesive, stiffening agent
Dextrose	Sweetening agent, binder
Ethylcellulose	Binder, coating material
Guar gum	Binder
Hydroxyethylcellulose	Binder, film former
Hydroxypropylcellulose	Granulating agent, binder
Hydroxypropylmethylcellulose	Film former in sustained release formulations
Hydroxypropylmethylcellulose phthalate	Binder in preparation of granules with sustained release properties
Lactose	Diluent, filler
Mannitol	Carrier, lubricant
Methylcellulose	Binder
Microcrystalline cellulose	Binder, diluent, disintegrant
Starch	Diluent
Sucrose	Sweetener

1.1. Chiral excipients

Some excipients, used in pharmaceutical formulations such as micro-crystalline cellulose (Fig. 1.1) are chiral. The interaction of enantiomers of a chiral drug with chiral formulation additives may lead to formation of transient diasteomers with different physical and chemical properties. The resolution of enantiomers on columns packed with a chiral material is based on the same principal. Due to their helical structure, cellulose polymers are capable of discriminating between enantiomers through steric hindrance (Dappen et al., 1986). Cellulose triacetate was first used by Hesse and Hagel (1976) for chiral separation. It has been suggested that the resolution of enantiomers with an aromatic ring may be achieved by the formation of weak bonds (such as hydrogen bonds) and inclusion of the aromatic ring(s) in crystalline acetyl cellulose in the swollen state.

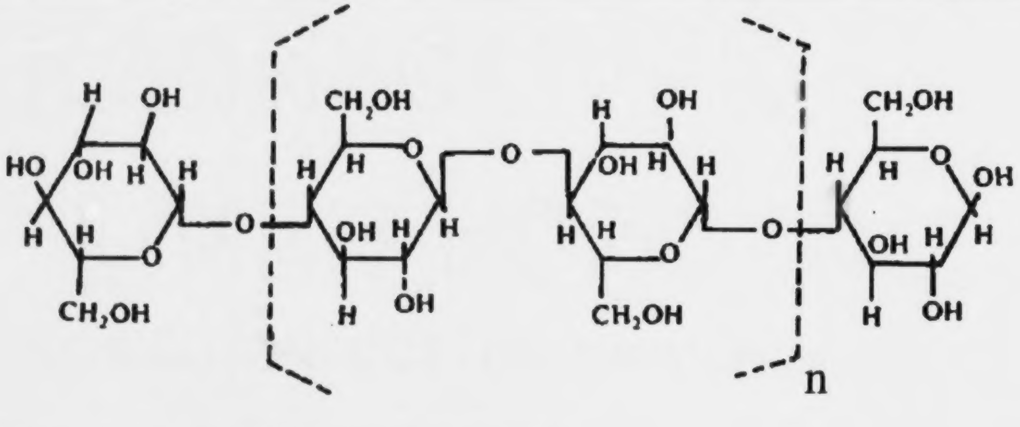

Figure 1.1. Structure of microcrystalline cellulose.

Table 1.2. Examples of chiral drugs whose enantiomers were resolved on a cyclodextrin stationary phase (adapted from Daniel et al., 1986).

Drugs	Resolution[a] value
β-adrenergic blockers	
Propranolol	1.40
Metoprolol	0.90
Antihistamine	
Chlorpheniramine	1.51
Calcium channel blockers	
Verapamil	0.71
Nisoldipine	0.87
Nimodipine	1.10
Diuretic	
Chlorthalidone	1.95
Sedative-anticonvulsants	
Hexobarbital	1.51
Mephobarbital	1.60
Mephenytoin	1.83
Triazoline	1.50
Phensuximide	1.54
Anticorticosteroid	
Aminoglutethimide	0.91
Nonsteroidal antiinfalmmatory drugs	
Ketoprofen	1.24
Narcotic analgesic	
Methadone	0.81

[a] Resolution is the measure of the distance between the two adjunct peaks in a chromatogram ($R=2\Delta T/W_1+W_2$; where T is retention time and W is peak width)

Cyclic oligosaccharides such as cyclodextrins are also used in the chromatographic resolution of enantiomers (Table 2.1) either as chiral mobile phase additives (Cabrera *et al.*, 1990) or chiral stationary phases (Dappen *et al.*, 1986). The mechanism by which cyclodextrins achieve their enantio-discrimination involves formation of inclusion complexes, with different strengths, for the individual enantiomers. Since chiral glucose units form the cyclodextrin cavity, the molecule acts as a chiral discriminator. The formation of hydrogen bonds between the individual enantiomers and the 2- and 3-hydroxyl groups located at the mouth of the cyclodextrin cavity is particularly important in chiral recognition (Daniel et al., 1986).

Indeed, the possibility of stereoselectivity in release of enantiomers from formulations containing racemic propranolol [excipient: hydroxypropylmethylcellulose (HPMC)-sodium alginate] (Duddu *et al.*, 1993) and verapamil (excipient: alginate and sugar spheres in a shellac barrier) (Aubry *et al.*, 1993) has recently been demonstrated.

In light of the fact that the pharmacological activity of enantiomers of chiral drugs may vary quantitatively as well as qualitatively, a change in the relative absorption of the enantiomers secondary to stereoselective release may influence the PD properties of the drug. Consequently, the time course of the pharmacological and/or toxicological effects may also be altered. Hence, a careful examination of *in vitro* and *in vivo* disposition pattern of a chosen class of drugs (e.g. 2-APAs) may shed light on the possibility of a stereoselective interaction between chiral excipients and enantiomers of racemic drugs such as tiaprofenic acid.

1.1.1 Cyclodextrins

Cyclodextrins are products of enzymatic degradation of starch. Starch consists of two main polymeric structures, branched amylopectin and linear amylose. The building blocks of these poly-sugars are D-glucopyranoside units linked by α-1,4 and α-1,6 glycosidic bonds. The partial degradation of amylopectin and amylose results in heterogeneous, water absorbing, and water soluble compounds called dextrins. Further simultaneous enzymatic degradation and the attachment of two ends of the dextrin molecules produces a cyclic oligosaccharide, cyclodextrin. The enzymes (cyclodextrin-glycosyl-transferases) involved in last step of the cyclodextrin production can be obtained from bacterial sources such as *Bacillus macerans*. Due to steric restrictions the enzymatic degradation of dextrins gives only three stable cyclodextrins (α-, β-, and γ-cyclodextrins) (Szejtli, 1982 and 1994). Cyclodextrins are cyclic oligosaccharides that consist of 6, 7, or 8 glucose units for α-, ß-, or γ, cyclodextrins, respectively, linked with α-1,4-glycosidic bonds (Saenger, W., 1984) (Fig. 1.2).

β-Cyclodextrin, and its more water soluble derivatives, in particular, have recently been widely utilized in the pharmaceutical formulation of various drugs (Rainsford, K.D., 1990). Specifically, the unique structure of cyclodextrins enable them to form a host-guest complex by accommodating a wide variety of drug molecules inside the hydrophobic cavity (Szejtli, 1984).

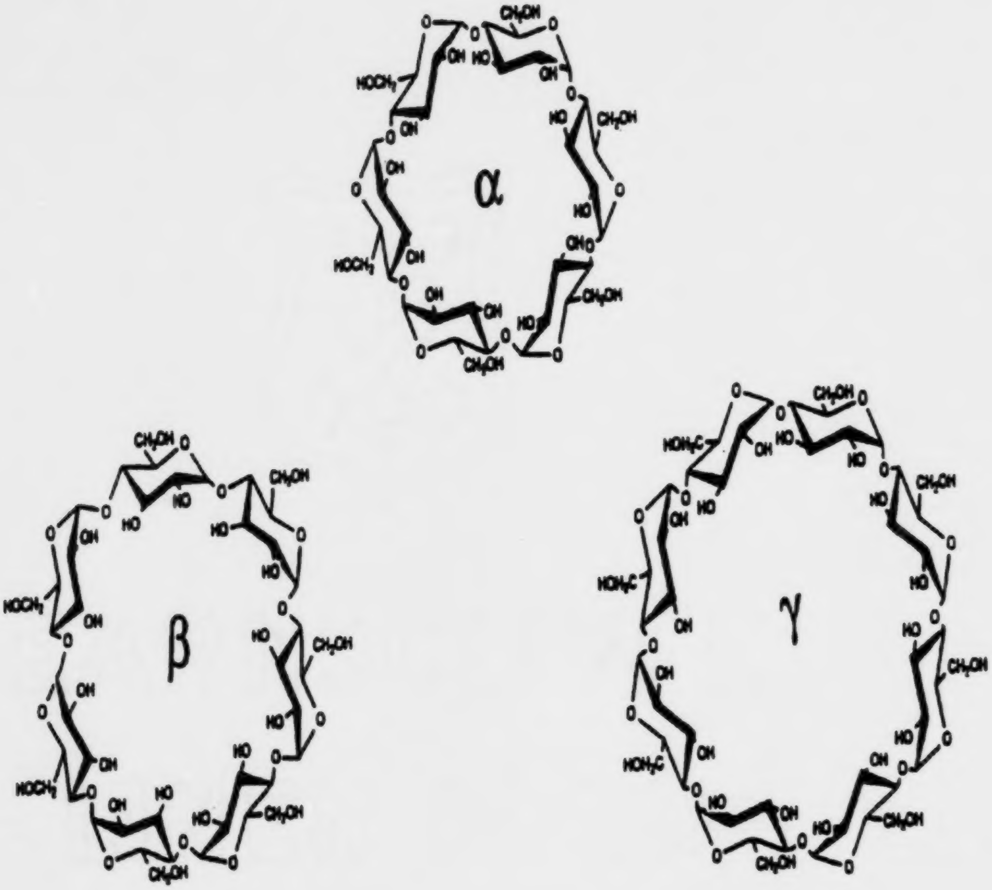

Figure 1.2. Structures of cyclodextrins.

1.1.1.1 Structural Modification of cyclodextrins

Numerous cyclodextrin derivatives can be prepared using chemical or enzymatic reactions. In β-cyclodextrin, which consists of 7 glucose units, there are 21 hydroxyl moieties. These hydrogen atoms or hydroxyls can be substituted with a variety of groups such as alkyl, hydroxyalkyl, and carboxyalkyl (Lindberg *et al.*, 1991, Irie *et al.*, 1989, Szejtli, 1980, 1994). The reasons for structural modifications of parent cyclodextrins can be summarized as:

- Improvement in the solubility of parent cyclodextrins;
- Improvement in the ability of molecules to form stable inclusion complexes;
- Attachment of catalytic groups (enzyme modeling);
- Formation of less soluble or insoluble derivatives of parent molecules (sustained release formulation and chromatographic purposes).

Glycopyranose units of the cyclodextrin molecule have three free hydroxyl groups. The relative reactivity of C(2) and C(3), secondary, and the C(6) primary hydroxyls differs depending on pH, temperature, and reagents employed in the reaction. The large scale production of modified cyclodextrins is limited due to the expense and generation of toxic waste material during modification and purification processes (Deegee, *et al.*, 1993). The lack of toxicological data on these derivatives reduces their pharmaceutical applicability (Szejtli, 1994). Only a few methylated and hydroxypropylated cyclodextrins have been widely used for pharmaceutical purposes.

Methylated β-cyclodextrin, the heptakis-(2,6-di-O-methyl)- and heptakis-(2,3,6-tri-O-methyl)-β-cyclodextrins (DMCD and TMCD, respectively) are more hydrophobic than the parent β-cyclodextrin molecule. Methylated β-cyclodextrins exhibit high aqueous solubility (Imai, 1988). DMCD has high solubility in cold water and is insoluble in hot water resulting in ease of purification and isolation. DMCD has been extensively studied as a solubilizing agent for poorly water soluble drugs (Szejtli, 1994).

Hydroxypropyl-β-cyclodextrin (HPCD) is more hydrophilic than β-cyclodextrin., It may therefore be used to enhance water solubility of drugs. Recently, considerable attention has been paid to the utilization of this derivative of β-cyclodextrin in various pharmaceutical formulations, particularly as a solubilizing agent in parenterals (Yoshida *et al.*, 1989).

In contrast to methylated and hydroxypropylated β-cyclodextrins, which have amphiphilic characteristics and high aqueous solubilities (Imai, 1988), ethylated ß-cyclodextrin (Fig. 1.3) is a hydrophobic compound and exhibits very low water solubility. It may, therefore, be used in the formulation of sustained release dosage forms due to its ability to reduce the rate of release and subsequent dissolution of water soluble drugs. Indeed, the feasibility of the preparation of a slow release formulation of diltiazem HCl, by forming an inclusion complex with diethyl-ß-cyclodextrin (DCD), has been previously demonstrated (Horiuchi *et al.*, 1990, Hirayama, 1993).

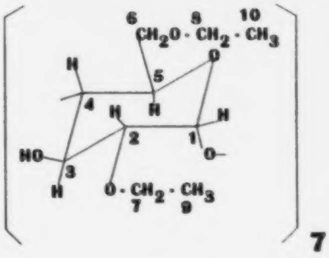

Figure 1.3. Structure of 2,6-diethyl-β-cyclodextrin

1.1.1.2. Physical and chemical properties

Some physicochemical properties of various cyclodextrins are depicted in Table 1.3 (Yoshida *et al.*, 1989, Hirayama *et al.*, 1993, and Szejtli, 1982 and 1994). The aqueous solubilities of parent cyclodextrins are dependent on temperature, the higher the temperature, the greater the solubility (Szejtli, 1982). A serious barrier to wider pharmaceutical utilization of β-cyclodextrin is its limited solubility. This problem, however, can be overcome by chemical or enzymatic modifications of parent cyclodextrin molecules. For example, methylated and propylated β-cyclodextrins show a significant increase in solubility compared to that of β-cyclodextrin. However, the di-ethylation of β-cyclodextrin at 2- and 6- hydroxyl positions results in a significant and substantial decrease in water solubility of parent oligosaccharide molecules (Hirayama *et al.*, 1993).

Native cyclodextrins have no reducing end groups, therefore, they give positive reactions in tests characteristic of this type of carbohydrate (e.g. color reaction with anthrone). They are not sensitive to alkali hydrolysis and have no well-defined melting point. They start to decompose above 200°C.

Partial hydrolysis of cyclodextrins to glucose units and linear oligosaccharides can be achieved using strong acid solutions (e.g. 1-5 M HCl). The hydrolysis of cyclodextrins is slower in the initial phase (corresponding to the opening of the cyclic structure) than the final phase (corresponding to hydrolysis of the linear oligosaccharide). For example, at 60°C in the presence of 1.15 M HCl β-cyclodextrin is rapidly hydrolyzed with $t_{1/2}$ values of 2.6 min and 0.9 min for the initial and final phases, respectively (Szejtli, 1982).

Table 1.3. Some physicochemical properties of cyclodextrins.

Cyclodextrins	Number of glucose units	Average Molecular weight	Solubility (g/100 mL)	Surface tension (mN/m)
α-Cyclodextrin	6	972	14.5	----
β-Cyclodextrin	7	1135	1.8	71
γ-Cyclodextrin	8	1297	23.2	----
DMCD	7	1331	>50	68
HPCD (degree of substitution: ~4.6)	7	1384	>33	71
DCD	7	1527	3.4×10^{-3}	53

1.1.1.3. Toxicity of cyclodextrins

Renal damage is the main toxicity following iv administration of β-cyclodextrin due to particularly high affinity to cholesterol (Szejtli, 1982). Parenterally administered β-cyclodextrin forms insoluble cholesterol-β-cyclodextrin complex in the kidneys causing nephrotoxicity. Methylated β-cyclodextrin-cholesterol complexes are more stable than native β-cyclodextrin-cholesterol complexes. Methyl-β-cyclodextrin is capable of extracting cholesterol from the erythrocyte membrane often resulting in extensive hemolysis. Nephrotoxicity is significantly reduced or abolished with modified cyclodextrins with higher water solubility such as HPCD (Szejtli, 1982 and 1994 and Yoshida *et al.*, 1989).

In contrast to iv administration, however, oral administration of β-cyclodextrin does not cause any apparent damage in the kidneys, GI tract, central nervous system, and/or blood cells. This is likely due to the lack of absorption of intact β-cyclodextrin and its derivatives from the GI tract.

1.1.1.4. Inclusion complexation of drug molecules with cyclodextrins

Inclusion complexs are products of complete or partial encapsulation of a guest molecule with a second host molecule. Cyclodextrins can, therefore, form inclusion complexes with a wide range of drug molecules having one or two aromatic rings or even larger molecules carrying a side chain (Szejtli, 1982 and 1994). Cyclodextrins have a truncated cone shape with a wider side formed by the secondary hydroxyl groups and the narrower side by primary hydroxyls (Fig. 1.2). Cyclodextrins provide a microheterogeneous environment since the exterior of the molecule is hydrophilic while the cavity is hydrophobic due to the relatively high electron density (Saenger, 1984). The cavity volumes of α-, β-, and γ-cyclodextrins per 1 mol formed by 104, 157, and 256 mL, respectively. The internal diameter of the β-cyclodextrin cavity is 7.8 °A which is suitable for encapsulation of drug molecules. In an aqueous environment the hydrophobic cavity of β-cyclodextrin molecule is occupied by water molecules. The interaction of water with the cavity of the β-cyclodextrin is thermodynamically undesirable due to polar-apolar interactions. Water molecules can readily be replaced by more hydrophobic drug molecules resulting in the formation of β-cyclodextrin-drug inclusion complexes.

The preparation of inclusion complex of drug molecules with cyclodextrins is a simple process. The most common method of preparation is to stir or shake an aqueous solution of cyclodextrin with a drug as solid particles (suspension) or in solution. Following establishment of equilibrium, water can be removed by freeze-drying, spray drying or by any other suitable method (Szejtli, 1982).

Another method for the preparation of an inclusion complex as a solid product is called kneading. This method involves kneading cyclodextrin and the drug with a small amount of water. Since the cyclodextrin-water interaction is thermodynamically unfavorable, the inclusion complex with drug is easily obtained. The preparation of cyclodextrin complexes of NSAIDs has been previously demonstrated (Kurozumi et al., 1975).

The formation of an inclusion complex is confirmed by X-ray crystallography, spectroscopic methods (IR and ESR spectroscopy), chromatography (thin-layer), and thermal analysis with a differential scanning calorimeter (Szejtli, 1982).

1.1.1.4.1. Cyclodextrin inclusion complexes and improved solubility and dissolution rate of drugs

There are numerous reports indicating significant improvement in the solubility and dissolution profiles of various NSAIDs. Using cyclodextrins Chow *at el.* (1986) have reported a 1:1 molar ratio complexation between ibuprofen and β-cyclodextrin which results in approximately a 12-fold increase in the solubility of the drug. In addition, the release and dissolution of ibuprofen was significantly enhanced by complexation (Chow *et al.*, 1986). In spite of hosting hydrophobic molecules, the cyclodextrin complexes are hydrophilic in nature. In an aqueous environment the hydrophilic outer surface of the cyclodextrin molecules comes in to direct contact with water molecules. This interaction results in an increase in the water solubility of the hydrophobic guest molecule. Following inclusion complexation with HPMC, a similar improvement in the solubility and dissolution characteristics of ketoprofen, naproxen and tenoxicam have been observed (Loftsson *et al.*, 1993). The net effect of complexation of a NSAID with hydrophilic cyclodextrin derivatives is to enhance water solubility and dissolution rate especially at low pH values (i.e. pH 2) (Masuda *et al.*, 1984, Tarimci and Celebi, 1988, Uekama *et al.*, 1981 and 1985, and Zecchi *et al.*, 1988).

Cyclodextrin can also be used to decrease solubility and dissolution rate of drugs. The hydrophobic derivatives of cyclodextrin such as DCD can be used to prepare slow release oral formulations. Following complexation with DCD, the release rate of diltiazem has been significantly reduced. The release rate of diltiazem from a DCD inclusion complex was not affected by pH, agitation rate, and low concentration of surfactant. This indicates the suitability of DCD as an excipient in the formulation of oral sustained release dosage forms (Horiuchi, 1990)

Systemic absorption of drugs from the GI tract involves a series of successive rate processes: disintegration of the dosage form and subsequent release of the drug, dissolution of the drug in the acidic environment of the stomach or at higher pH in the duodenum and small intestine, and absorption across the GI membranes into the systemic circulation. The overall absorption rate of the drug from the GI tract is rate-limited by the slowest step mentioned above. For drugs with poor water solubility (e.g. NSAIDs), the rate-limiting step is the rate at which the drug dissolves (dissolution) (Levy *et al.*, 1966).

Inclusion complexation with cyclodextrins is characterized by a significant improvement in the bioavailability of drugs. This can be attributed to the enhanced solubility and dissolution rate of the drug which is partially or completely included in the cavity of the cyclodextrin molecule. In humans, the time of the attainment of peak concentration (t_{max}) for fendiline HCl was reduced from 2 h to 15 min upon complexation with β-cyclodextrin (Szejtli, 1994). Similarly, a significant improvement in the absorption rate of ibuprofen and flurbiprofen (FB) upon complexation with β-cyclodextrin and its derivatives has been demonstrated (Table 1.4) (Chow *et al.*, 1986, Loftsson *et al.*, 1993, Otero-Espinar *et al.*, 1991).

Table 1.4. Bioavailability and cyclodextrins

Compound	**Species**	**$AUC_{0\text{-}8}$ (μg•h/L)**	**Cmax (μg/mL)**	**t_{max} (h)**
Flurbiprofen (50 mg)	Rabbits	73.9	14.2	2
Flurbiprofen-β-cyclodextrin (equivalent to 50 mg flurbiprofen)	Rabbits	137	37.6	1
Flurbiprofen-TMCD (equivalent to 50 mg flurbiprofen)	Rabbits	160	43.0	1
Ibuprofen (300 mg)	Rat	311	120	0.92
Ibuprofen-β-cyclodextrin (equivalent to 300 mg ibuprofen)	Rat	321	112	0.37

As demonstrated by Horiuchi *et al.* (1990) hydrophobic cyclodextrin, ethylated derivatives, and the mixture of these derivatives with parent β-cyclodextrin may be successfully used to retard the rate of drug release. The oral administration of tablets containing a DCD-diltiazem complex to dogs resulted in a prolonged concentration of the drug in plasma with higher plasma concentrations at 12 and 24 h as compared to those observed after diltiazem tablets (Horiuchi *et al.*, 1990).

1.1.1.4.2. Influence of cyclodextrins on chemical and physical stability of drugs

The ability of cyclodextrin to encapsulate drug molecules makes them valuable agents for improvement of the physical and chemical stability of unstable drug molecules. Cyclodextrin can decelerate or accelerate various kinds of reactions in solution including oxidation, hydrolysis, decarboxylation, and isomerization. Following complexation with β-cyclodextrin, the rate of alkaline hydrolysis of p-amino-benzoate was reduced. It seems that cyclodextrins (β-cyclodextrin, HPCD) have a stabilizing effect on acetylsalicylic acid, melphalan, cholecalciferol, prostaglandin E_1, digitoxin, and thalidomide, in aqueous solution (Szejtli, 1994, Wiese *et al.*, 1991, Yosida *et al.*, 1988, Krenn *et al.*, 1992).

Cyclodextrin may also affect the racemization of the enantiomers of chiral drugs. They (except for α-cyclodextrin) retarded the chemical racemization of both (-)-S-hyoscyamine and (-)-S-scopolamine. It has been suggested that the inclusion of drug molecules in cyclodextrins may inhibit the attack by hydroxyl ions and/or water molecules and thus decreases the rate of the racemization reaction (Blaschke *et al.*, 1993).

1.1.1.4.3. Improved pharmacological and toxicological profiles of drugs

Crystals of sparingly soluble drugs (e.g. NSAIDs) may remain in contact with the gastrointestinal (GI) mucosa resulting in high local drug concentrations which may induce and/or aggravate NSAID-induced GI damage (Szejtli, 1994). Indomethacin, flurbiprofen (FB), biphenylacetic acid, naproxen, and phenylbutazone are examples of drugs with GI mucosal irritating effects that may be reduced by cyclodextrin complexation (Szejtli, 1994, Rainsford, 1990, Lee *et al.*, 1994). However, the focus of all the above studies is the reduction of upper GI toxicity while the possibility of damage to the more distal segments of the intestine have been overlooked. It has been demonstrated that NSAID-induced GI toxicity is not confined to the gastroduodenal region and serious complications may occur in the lower intestine as well (Rainsford, 1990). It is now evident that any assessment of NSAID formulations should include both the upper and the lower GI tract.

The complexation of drugs with cyclodextrin may also improve the desired pharmacological activity of drugs. Epidural and intrathecal administration of fentanyl-like opioid inclusion complexes with HPCD resulted in a potentiation of spinal activity, (Hoshino et al., 1989). It has been suggested that dipyridamol-β-cyclodextrin complex causes a stronger coronary vasodilatation in dogs, at doses much lower than those required to influence the systemic arterial pressure and heart rate. *In vitro* platelet aggregation induced by adenosine diphosphate was inhibited by complexation of dipyridamol with β-cyclodextrin (Fregnan *et al.*, 1990).

1.1.1.4.4. Application of cyclodextrin in Pharmaceutical dosage forms

In recent years, cyclodextrin and its derivatives have gained wide-spread use in the formulation of various pharmaceutical dosage forms (oral, solid, liquid, and parenteral formulations). In addition, numerous studies indicate the potential application of cyclodextrins in the formulation of rectal, pulmonary, transdermal, and nasal drug delivery systems (Szejtli, 1994). Furthermore, cyclodextrins are emerging as an excipient in tablet technology. In compressed tablets β-cyclodextrins may be incorporated as an inclusion complex (stabilizing or solubility enhancer of active ingredient) or as a filler and/or binder due to their ability to improve compression properties of powder blend. Cyclodextrins have been studied as excipients in the tablet formulations of many therapeutically important drugs such as acetylsalicylic acid, ergocalciferol, isosorbide 5-monohydrate, phenytoin, sulfamethoxazole, and furosemide (Szejtli, 1994, Kata, *et al.*, 1990).

1.1.1.5. Cyclodextrins and enantiomeric resolution

Cyclodextrins are optically pure compounds capable of acting as chiral selectors. These cyclic molecules are extensively used for the resolution of enantiomers of therapeutically important compounds (Table 1.2). The drug molecules fit in the cavity of the cyclodextrins and resolution occurs due to formation of bonds (hydrogen, hydrophobic etc.) between the substituted groups on the outside of the cyclodextrin cavity and the drug molecule. It has been suggested that modified cyclodextrins may exhibit a higher degree of chiral selectivity due to additional electrostatic interactions between the chiral drug and the groups at the entrance of the cyclodextrin cavity (Lipkowitz *et al.*, 1993).

Imai (1988) and Lipkowitz (1993) have reported stereoselective interaction of 2-APA with cyclodextrins. S-flurbiprofen is preferentially included in the cavity of trimethyl-β-cyclodextrin (Imai *et al.*, 1988). This interaction has been attributed to changes in the capacity for intramolecular hydrogen bonding due to the replacement of the hydroxyl moieties by methoxy groups (Imai *et al.*, 1988). In a recently published study, the difference in the binding enthalpy, (ΔH), was determined for the individual enantiomers of fenoprofen, flurbiprofen, 1-phenylethanol, and mandelic acid. An enhanced enantioselectivity in favor of the S enantiomer of the aforementioned drugs (high difference in binding enthalpy values) was observed when the alkylated cyclodextrins were employed as host molecules. These differences in enantio-discriminating ability of derivatized vs native cyclodextrins has been attributed to a significant intermolecular contact with the exterior of the derivatized cyclodextrins. On the other hand, there is only interior contact between the drug and the native cyclodextrin molecule. Therefore, it seems that chiral discrimination may also take place on the exterior of cyclodextrins (Lipkowitz *et al.*, 1993).

1.1.1.5.1. Cyclodextrins and stereoselective release

The possibility of stereoselective release and subsequent absorption of the enantiomers may occur when cyclodextrins are used in the formulation of racemic drugs. However, a rapid rate of dissolution may render detection of stereoselectivity (if any) difficult. ß-Cyclodextrin, and its water soluble derivatives such as trimethyl-β-cyclodextrin, usually improve the dissolution characteristics of drugs such as NSAIDs, and thus enhance the rate of dissolution (Zecchi *et al.*, 1988, Imai *et al.*, 1985, and Orienti *et al.*, 1989). Alternatively, a reduction in the rate of release of enantiomers, by inclusion complexation with hydrophobic DCD, may provide suitable conditions for stereoselective intermolecular interactions and/or release.

1.2. Hydroxypropylmethylcellulose (HPMC) and ionotropicaly gelled sodium alginate system

Incorporating drugs in hydrophilic matrix systems (e.g., HPMC) offers an easy and reproducible approach to modification of drug release rate. The use of a matrix system to control drug release from the formulation offers several advantages such as the ease of preparation, and reduced probability of accidental release due to homogeneous dispersion of drug in the polymeric matrix (Grass 1990). In sustained release formulations containing HPMC, the mechanism for the retardation of the drug release involves the rapid formation of a gel layer around the surface of the matrix upon exposure to the GI fluid. It has been shown that the overall release of drugs from polymeric matrices is based on Fickian diffusional release and/or Case II matrix relaxation. Case II relaxation release is the drug transport mechanism associated with stresses and state-transition. (Peppas *et al.*, 1989, Duddu *et al.*, 1993, Ford *et al.*, 1991, Conte *et al.*, 1993).

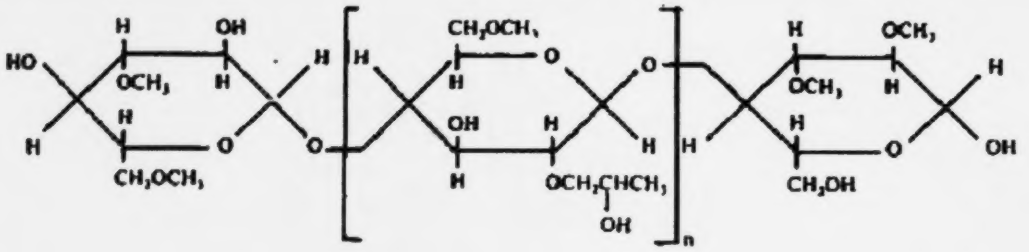

Figure 1.4. Structure of HPMC

1.2.1. Physical and chemical properties of HPMC

HPMC, cellulose hydroxypropylmethylether (Fig. 1.4), is an odorless, tasteless, white or creamy-white fibrous or granular powder. In general, HPMC can be categorized as high and low viscosity HPMC grades. It undergoes a reversible gel to solid state upon heating and cooling, respectively. Depending on the grade, the hydrated solution of HPMC has a pH range of 6 to 8. It is soluble in cold water, forming a viscous colloidal solution, but insoluble in alcohol, ether, and chloroform. It is a surface active agent in solution with surface tension ranging from 42 to 56 dynes/cm. HPMC is stable as a solution over a wide pH range (pH, 3-11). An aqueous solutions of HPMC are prone to bacterial growth and anti-microbial agents should be added for preservation. HPMC is non toxic in humans and animals (Boylan, 1986). Being a multiple-use excipient, HPMC, is utilized in tableting and sustained release technology as a wet binder (0-5% of formulation), film former (5-20% of formulation), and as a sustained release excipient (5-60% of formulation) (Grass, 1990).

HPMC has been used as a hydrophilic carrier system with sodium alginate or chitosan to prepare prolonged release multiple-unit dosage forms of NSAIDs such as ibuprofen and indomethacin (Bodmeier, 1991). HPMC-alginate beads can be prepared by ionotropic gelation of the polysaccharide. This method involves the entrapment of HPMC within a gel network formed via ionotropic gelation of the sodium alginate or chitosan with counterion (Bodmeir, 1991).

1.2.2. Stereospecific interaction and release of enantiomers of chiral drug from HPMC-sodium alginate matrix

HPMC is an optically pure water soluble chiral cellulose polymer. Hence, there is a possibility of stereoselective interaction between HPMC and the enantiomers of chiral drugs included in the formulation. The stereoselective release of propranolol enantiomers from a sustained release formulation consisting of HPMC-alginate system has been previously demonstrated (Duddu *et al.*, 1993). The observed stereoselectivity, although small, was statistically significant. Two types of release have been observed for propranolol enantiomers; sigmoidal and nonsigmoidal profiles with plateaus and burst effects. Sigmoidal release profiles exhibited a statistically significant difference between the two enantiomers of propranolol. In contrast, the characteristic feature of nonsigmoidal profiles was the lack of stereoselectivity accompanied with a burst effect. The observed stereoselectivity in the sigmoidal profiles has been attributed to a difference in the diffusivity of the two enantiomers through the chiral translucent hydrated layer next to the chiral matrix. Alternatively, it may reflect a difference in complexation constants of each enantiomer with the chiral excipients in the hydrated layer. It has been suggested that the release of a water soluble drug (e.g. propranolol HCl) from the matrix composed of HPMC occurs based on the following three successive processes:

1. Diffusion of water into the matrix causing its hydration;
2. Diffusion of drug through the hydrated chiral matrix (possibly stereoselective);
3. Erosion of the hydrated matrix (nonstereoselective).

Moreover, the lack of stereoselectivity in irregular nonsigmoidal release profiles highlights the importance of the diffusion process in the preferential interaction and/or release of propranolol enantiomers (Duddu *et al.*, 1993). Therefore, when assessing dissolution of racemic drugs, the possibility of intermolecular interaction between the enantiomers and chiral excipients and subsequent stereoselectivity in the release must be considered.

1.3. Liposomes

Liposomes are composed of phospholipid that spontaneously form unilamellar or multilamellar concentric bilayers separated by aqueous compartments. Since their discovery by Bangham (1965), liposomes have emerged as important drug delivery systems (Fildes, 1981). They may offer several advantages as drug carrier systems including:

- Entrapment of both polar and nonpolar drugs;
- Retention of the entrapped molecule for an extended time period;
- Increased transport of drug molecules through biological membranes;
- Targeting of drug molecules to specific cells.

Liposomes, as a drug delivery system, are well established. In recent years, considerable attention has been paid to the delivery of anticancer agents, antifungal drugs, antibacterials, antivirals, and antiparasitics using liposomal carrier systems (Cullis, 1987). Liposomes may also improve the toxicological profile of drugs. It has been suggested that the association of NSAIDs with exogenous zwitterionic phospholipids may reduce GI toxicity of these drugs by preventing the reduction of hydrophobic barrier properties of the mucous gel layer caused by NSAIDs (Lichtenberger, 1995).

1.3.1. Structure and preparation of liposomes

Similar to biological membranes, the main structural components of liposomes are phospholipids. Phospholipids, such as phosphatidylcholine, are amphiphilic molecules. The chemical structure of phospholipids is based on a glycerol backbone which is esterified at both the C(1) and C(2) positions. The fatty acids occupying both C(1) and C(2) positions may be the same (symmetric structure) or different (asymmetric) (Eibl, 1981).

Although phospholipids possess both polar and apolar tips, they are not soluble in water. In an aqueous environment, however, phospholipid molecules align themselves in such a manner that their hydrophilic groups face the bulk aqueous phase. The planar bilayer arrangements of phospholipid molecules minimizes unfavorable interactions between the polar water molecules and apolar hydrocarbon fatty acid chains.

Subsequently, the bilayer sheets fold on themselves further reducing and/or eliminating undesirable hydrophilic-hydrophobic interactions (Bangham, 1981, Gruner, 1987).

Phospholipid bilayer characteristics such as gel- to -liquid crystalline transition, permeability, partition coefficients, electrical properties, and elastic properties are important in liposome technology. These properties are altered by using mixtures of phospholipids (neutral and charged). Furthermore, including cholesterol in the bilayer may increase or decrease the bilayer fluidity based on the physical state of the liposome (Gruner, 1987). Liposomes may be prepared from phospholipids employing a number of techniques such as hydration, reverse-phase evaporation, detergent removal, and solvent injection methods. In general, the method used in the preparation of liposomes determines their size and type. Liposomes are classified, based on their size, into 4 types (Eibl, 1981):

- Multivesicular vesicles (MVVs) (1 µm to 10 µm);
- Multilamellar vesicles (MLVs) (size: 100 nm to ~10 µm);
- Large unilamellar vesicle (LUVs) (size: 50 nm to 1 µm);
- Small unilamellar vesicles (SUVs) (size: 10 nm to 50 nm).

1.3.2. Stability of liposomes

The physical integrity of liposomes can be studied by evaluating the leakage of an entrapped solute Following mixing of a detergent solution with liposomes, the changes in the turbidity of the mixture can be monitored using a spectrophotometer operated in the visible region. A decrease in the degree of turbidity of the mixture indicates damage to the liposomal integrity (Regan *et al.*, 1980 and Defrise-Quertain *et al.*, 1985). At high temperatures and at extreme high pH values phospholipids undergo hydrolysis of the fatty acid attached to the C(2) position of the glycerol moiety producing lysophospholipid and free fatty acid. The formation of such hydrolysis side products significantly increases the liposomal permeability (Grit *et al.*, 1989).

After *in vivo* administration, liposomes are susceptible to the catalytic activity of serum proteins [high density lipoporteins (HDLs)], various proteases and peptidases, bile salts, and changes in pH (Op Den Kamp *et al.*, 1974 and Richards *et al.*, 1978). Liposomes administered iv are rapidly taken up by the reticulo-endothelial system (RES) (e.g. liver, spleen, lung, blood cells, and bone marrow). The uptake of liposomes by RES is the main obstacle in their wider medicinal application. Over the years, this drawback of liposomes (i.e., RES uptake) has been gradually overcome. Prolonged circulating liposomes have been produced by either mimicking the outer composition of red blood cells by the inclusion of sialic acid moieties, or stabilizing the lipsomes by steric forces through the inclusion of phospholipids derivatized with synthetic hydrophilic polymer headgroups in the bilayer (Allen and Chonn, 1987, Nucci *et al.*, 1991) .

1.3.3. Optically pure polysaccharide-coated liposomes and stereoselective release

Coating liposomes with polysaccharides can improve the stability of liposomes against enzymatic hydrolysis and disruption of liposomal structure by bile salts. The chiral and optically pure polysaccharides such as O-palmitoylpullulan, cholesterolpullulan, and carboxymethylchitin are used for coating liposomes (Sato *et al.*, 1992, Dong *et al.* 1992). Polymer molecules can be adsorbed on the surface of the liposomes by hydrophilic or ionic forces (Seki *et al.*, 1984). In addition, polymer molecules can be fixed on the surface of the liposomes via hydrophobic anchors penetrating the phospholipid bilayer (Sunamoto *et al.*, 1989).

Liposomes can entrap both hydrophilic and hydrophobic compounds. In general, hydrophobic compounds tend to remain in the phospholipid bilayer while hydrophilic molecules reside in the aqueous compartment of the liposomes vesicle. The release of chiral drugs from polysaccharide-coated liposomes may exhibit stereoselectivity due to the preferential interaction of the individual enantiomer with the chiral coating polymer. The stereoselective release of the enantiomer may become particularly apparent after coating the surface of the liposomes with optically pure chiral molecules due to significant reduction in the release rate. This may provide sufficient time for the interaction of enantiomers with chiral excipient(s) included in the liposomal formulation and/or chiral polymeric coating agent.

1.4. Nonsteroidal antiinflammatory drugs (NSAIDs)

Nonsteroidal antiinflammatory drugs (NSAIDs) have an asymmetric centre and exist as an equal proportions of two enantiomers, R and S. Most of these chiral NSAIDs are administered in their racemic form, except for naproxen. *In vitro* studies have shown that the antiinflammotry effect is mainly due to the S enantiomer (Caldwell *et al.*, 1988 and Williams and Day 1988). The R enantiomer can, however, exhibit analgesic properties as demonstrated with flurbiprofen (Brune *et al.*, 1991).

In this study tiaprofenic acid (TA) and flurbiprofen were chosen as model drugs. Since the majority of the work was carried out using TA a detailed description is provided below. A more detailed review of flurbiprofen can be found elsewhere (Berry, 1993)

1.4. Tiaprofenic acid

Tiaprofenic acid (TA) is a member of the 2-APA class of NSAIDs with potent prostaglandin inhibitory activity (Deraedt *et al.*, 1982). Tiaprofenic acid entered the international market in 1976 and a decade later its use was approved in Canada. Tiaprofenic acid is as effective as the other commonly used NSAIDs in the treatment of osteoarthritis, rheumatoid arthritis, acute gouty arthritis, and ankylosing spondylitis (Camp *et al.*, 1981; Wojtulewski *et al.*, 1981). Tiaprofenic acid is also indicated in the relief of post-operative pain (Scarpa *et al.*, 1988), and in migraine attacks (Pini *et al.*, 1990) owing to its potent analgesic effects. TA is commonly administered as 200 mg tablets or capsules for the treatment of inflammatory conditions. A sustained release preparation of TA has recently been developed which may improve patient compliance (Essigman *et al.*, 1987). However, its therapeutic advantage over regular release formulation is not unequivocally proven. Several studies were not able to demonstrate a direct relationship between dose or concentration and desired therapeutic activities for TA (Bradley *et al.*, 1992; Day *et al.*, 1982; Laska *et al.*, 1986). The time course of desired effects (anti-inflammatory and analgesic) and their relationship with the concentration of the individual enantiomers of TA has not yet been explored.

TA possesses a chiral centre and is marketed as the racemate. The anti-inflammatory activity is mostly attributed to the S enantiomer (Jamali *et al.*, 1989). Several detailed studies about the pharmacological and therapeutic applications of TA have been published (Sorkin and Brogden 1984; Deraedt et al 1982), all of which ignore the chiral nature of the TA molecule.

1.4.1. Chemistry of tiaprofenic acid

Tiaprofenic acid, [(±)-5-benzoyl-α-methyl-2-thiophenacetic acid]; $C_{14}H_{12}O_3S$ (C 64.68%; H 4.65%; O 18.45%; S12.32%) is a white microcrystalline powder with melting point about 92-95°C and pK_a value of 3.0 (Fig 1.5).The molecular weight of TA is 260.16. It is soluble in ethanol, chloroform, and alkaline solutions. Its UV spectrum exhibits a shoulder at 260 nm and maximum wavelength at 305 nm in aqueous solutions (Koppel *et al.*, 1984 and Merck index 1983).

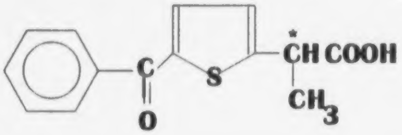

Figure 1.5. Structure of tiaprofenic acid (* denotes chiral centre)

1.4.2. Analytical methods

There are numerous analytical methods for the quantification of TA in biological samples; these include thin-layer, spectrophotometric, differential polarographic, gas chromatographic (GC), and high performance liquid chromatographic (HPLC) (Table 1.5).

The earlier reported assays of TA are nonstereospecific (Pottier *et al.*, 1977, Mohmed *et al.*, 1984). In 1986 Singh *et al.* developed the first stereospecifc GC assay for

TA. Mehvar *et al.* (1988) have reported a sensitive HPLC technique which allows for the quantification of individual enantiomers of TA in plasma and urine. This method involves formation of diastereomers through reaction of the carboxylic acid moiety with a coupling reagent, 2,2,2-trichloroethyl chloroformate, to form a mixed anhydride, followed by formation of an amide using L-leucinamide. The obtained amide diastereomers of TA were resolved on an achiral column. Although the importance of stereoselectivity in the pharmacokinetics of chiral drugs is well established, several nonstereospecific assays of TA have still published in recent years (e.g. Riek and Platt, 1987).

1.4.2.1. Enantiomeric conversion

It has been previously reported that in humans TA exhibits non-stereoselective pharmacokinetics using indirect stereospecific HPLC assays [pre-column derivatization using L-leucinamide or S(+)-amphetamine sulfate] (Mehvar *et al.* 1988 and Singh *et al.*, 1986, respectively). A recent report suggests the possibility of the existence of a small but significant difference between pharmacokinetics of TA enantiomer in humans when a direct HPLC method was used for analysis of plasma samples (Muller *et al.*, 1993 and Hutt *et al.*, 1994).

Table 1.5. Chromatographic assays for tiaprofenic acid

Minimum quantifiable concentration (mg/L)	Assay type	Volume (mL)	Specimen	Reference
Nonstereospecific assays				
0.1	HPLC	1.0	Plasma/Urine	Marecek *et al*., 1981
0.5	HPLC	0.2	Plasma	Ward *et al*., 1982
0.5	HPLC	1.0	Plasma	Lucker *et al*., 1982
0.5	HPLC	----	Tablets	Mohamed *et al*., 1984
0.25	HPLC	0.2	Plasma/Urine	Jamali *et al*., 1984
0.6	HPLC	0.2	Plasma	Koppe *et al*., 1984
0.1	HPLC	0.2	Plasma	Nilsen *et al*., 1985
0.1	HPLC	0.5	Plasma/Urine	Rieck and Platt, 1987
Stereospecific assays				
0.075	GC	1.0	Plasma/Urine	Singh *et al*., 1986
0.2	HPLC	0.5	Plasma/Urine	Mehvar *et al*., 1988

The discrepancy between these reports may be explained by partial or complete chiral conversion during derivatization. Indeed, a limitation previously reported for indirect techniques is the possibility of enantiomeric conversion of the analyte during the derivatization reaction. This chemical conversion may be limited (3- 6% for flurbiprofen) (Wright *et al.*, 1993), or complete (100% for ketorolac) (Vakily *et al.*, 1995).

Unavailability of the pure enantiomers renders evaluation of enantiomeric interconversion complicated or even impossible. This may explain why the possibility of enantiomeric conversion was not properly addressed during the development of the indirect HPLC methods reported earlier (Singh *et al.*, 1986 and Mehvar *et al.*, 1988). However, due to recent technological advances, the optical purification of the individual enantiomers of chiral drugs has been simplified which makes the study of optical stability more feasible. The organic base which is used during the derivatization process, and the concentration of the ethyl chloroformate are important factors in the degree of enantiomeric conversion of NSAIDs with the mixed anhydride method. It has been demonstrated that the lower the concentration of ethyl chloroformate the higher the degree of enantiomeric conversion. On the other hand, some drugs such as ketoprofen and etodolac exhibit a high degree of optical stability during the pre-column derivatization process indicating the importance of molecular structure in optical stability of drugs (Wright *et al.*, 1993). Examination of highly enantiomerically enriched samples of TA, using the derivatization technique indicated the possibility of partial enantiomeric conversion (~15%) (Hutt *et al.*, 1994).

It is, therefore, extremely important to investigate the possibility of partial or complete enantiomeric conversion of the enantiomers when developing a new stereospecific assay regardless of method of resolution. Altered enantiomeric composition of the samples due to enantiomeric conversion renders the interpretation of pharmacokinetic studies complicated and may result in erroneous conclusions.

1.4.3. Human pharmacokinetics

1.4.3.1. Absorption

TA is available as oral, topical, intraocular, intravenous, intramuscular, and rectal formulations (Pottier *et al.*, 1982). It is, however, commonly given orally as regular release tablets (Surgam^R, Roussell) or as a sustained release preparation (Surgam SR^R, Roussel).

Following oral administration of single doses (200-300 mg) of regular release tablets, TA is rapidly absorbed, with time to peak plasma or serum drug concentrations (t_{max}) of 0.5-2 h (Jamali *et al.*, 1985 and Singh *et al.*, 1988). Stereoselectivity in the absorption of TA is not expected as it is absorbed by passive diffusion. Indeed, similar AUC, C_{max}, and t_{max} values were reported for TA in arthritic patients and in healthy subjects (Singh *et al.*, 1986).

After 200 mg three times a day multiple oral doses, the absorption characteristics of TA remained the same as those observed after a single dose of the drug. Although its rectal absorption was slow, the extent of absorption after both rectal and oral administrations was similar (Pottier *et al.*, 1982). The oral bioavailability of TA from the sustained release formulation was comparable to that observed after the regular release tablets. On the other hand, the rate of absorption was significantly lower after the sustained release product as indicated by a lower C_{max} and longer t_{max} values (Hosie and Hosie, 1987).

In the fasting condition, increasing the dose of TA from 200 mg to 300 mg resulted in a proportional increase in AUC values indicating linear PK for this drug. Concomitant food intake with 200 mg of TA did not affect t_{max} while the C_{max} and AUC values were significantly reduced (Nilsen *et al.* 1985 and Lucker *et al.*, 1982).

The effects of rheumatoid arthritis and migraine on the PK of TA were evaluated. The absorption of TA remained unchanged in rheumatoid arthritis and during and after migraine attacks (Daymond and Herbert, 1982, Daymond and Herbert, 1983 Pini *et al.*, 1990).

1.4.3.2. Distribution

The distribution of TA enantiomers is not stereoselective with identical apparent volume of distribution (V_d/F) values for both S-TA (17.24±13.07 L) and R-TA (17.05±12.99 L). In humans, tissue binding of TA is limited as indicated by its small V_d/F value (~0.25 $L\cdot kg^{-1}$) (Singh *et al.*, 1986 and Lin *et al.*, 1987). Similar to other NSAIDs, TA is extensively (>99%) bound to plasma albumin at normally observed therapeutic concentrations (Nilsen *et al.*, 1985).

Aging and diseases may alter not only elimination but also the distribution of drugs. For example, albumin levels which may influence drug distribution may be reduced in elderly subjects and in conditions of liver and renal function impairment (Svensson *et al.*, 1986). Aging does not appear to alter the protein binding of TA (Nilsen *et al.*, 1985)

(Nilsen *et al.*, 1985 and Jamali *et al.*,1988 and 1989). It has been reported, however, that the protein binding of TA is significantly decreased in the presence of renal dysfunction (50% increase in the free fractions). This has been attributed to the decreased concentration of albumin and accumulation of endogenous substances such as organic acids which compete with TA enantiomers for the same binding sites on the albumin molecule.

In healthy subjects and rheumatoid arthritic patients, TA penetrates into the synovial fluid (the proposed site of action of NSAIDs) and results in substantial accumulation of drug in this small compartment (Walls and Simkin, 1983 and Kurowski, 1988). Following administration of a single dose or multiple doses of TA, as regular or sustained release formulations, the initial concentrations of drug in synovial fluid were low and gradually increased and exceeded those observed in plasma at 3 to 4 h post-dose. Subsequently, following 200 mg three times a day, maximum concentration (5-12 μg/mL) is attained at approximately 4 h post-dose in synovial fluid. TA concentration remains relatively constant over an 8 hour period. At 2 h post-dose the plasma:synovial fluid ratio has been reported to be 2.5:1 which is changed to 1:2 at 12 h post-dose. (Daymond and Herbert 1982 and 1983, Strusberg *et al.*,1983, Nichol *et al.*, 1988, and Young *et al.*, 1991). Singh *et al.*, (1988) observed a similar trend in the plasma to the synovial fluid distribution of the two enantiomers of TA. The synovial fluid distribution of TA enantiomers was nonstereoselective.

1.4.3.3. Metabolism and excretion

After iv and oral administrations, the systemic (CL_s) and oral (CL/F) clearances of TA enantiomers were not significantly different with similar $t_{1/2}$ values for the two enantiomers (Pottier et al 1982, Mehvar *et al.*, 1988, and Singh *et al.*, 1986). In humans, 60-75% of each daily dose is excreted in urine as acylglucuronide conjugates with no difference between enantiomers. (Adams *et al.*, 1973, Jamali *et al.*, 1988). Renal clearance of intact TA represents approximately 5-10% of the CL_s (Lucker *et al.*, 1982). However, it has been suggested that TA is mainly metabolized via glucuronidation and a small amount (10% of administered dose) of the intact drug recovered in urine may be due to hydrolysis of unstable acylglucuronides in the bladder or during the sample preparation (Mehvar *et al.*, 1988). Additionally, it has been reported that 30-40% of the administered dose of TA was recovered in the feces (Szpunar *et al.*, 1987 and Pottier *et al.*, 1977).

Tiaprofenic acid is metabolized via oxidation to [5-(-p-hydroxybenzoyl)-α-methyl-2-thiophenacetic acid] and via reduction to [5-(α′-hydroxy-benzyl)-α-methyl-2-thiophenacetic acid]. Both metabolites of TA retain their chiral centres (Fig 1.6). The antiinflammatory activities of the oxidized and reduced metabolites are only 1 to 5% of the parent drug, respectively. Tiaprofenic acid and its metabolites undergo extensive conjugation with glucuronic acid, and subsequently, are excreted mainly into the urine (Adams *et al.*, 1975). Tiaprofenic acid does not undergo enantiomeric inversion in humans (Mehvar *et al.*, 1988; Singh *et al.*, 1986),

After the administration of 200 mg TA three times a day to healthy subjects, Jamali *et al.*, (1984) found a total of 52.6-73.5% of the dose excreted into urine. The acyl glucuronide conjugates of the parent molecule constituted 36.5-56.7%, the reduced metabolite 7.5-13.1%, and the oxidized metabolite 4.9-7.5% of the administered dose. Arthritic patients at steady state excreted 55.7-70.4% of the given dose (200 mg three times a day) as acyl glucuronide conjugates of TA (43.4-55.1%), reduced metabolite of TA (5.9-10.1%), and oxidized metabolite of TA (5.8-6.6%) (Jamali *et al.*, 1984). Singh et al. (1986) have demonstrated that the excretion rate of the conjugated TA enantiomers are similar and the cumulative amounts excreted into the urine are virtually identical. The stereochemistries of phase I metabolites of TA have not yet been determined but since <10% of TA is metabolized via this route, the contribution of these metabolites to the overall pharmacokinetics of TA is not expected to be substantial.

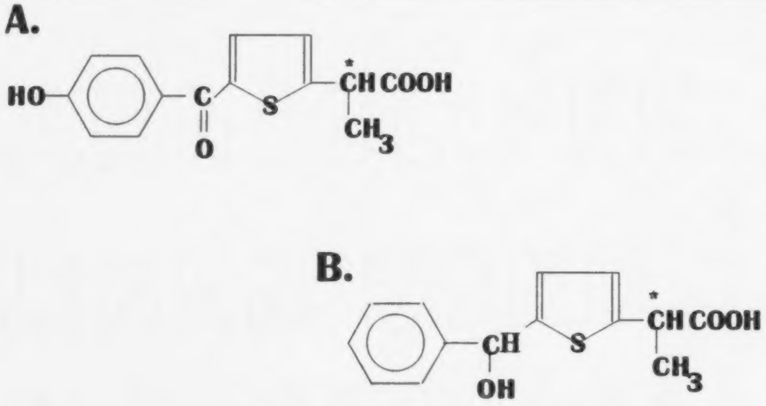

Figure 1.6. Phase I Biotransformation products of tiaprofenic acid. (* denotes chiral centre)

1.4.3.5. Effect of aging and disease on tiaprofenic acid pharmacokinetics

NSAID use in elderly subjects may lead to development of renal insufficiency (Lamy, 1987). In elderly subjects, TA does not appear to cause damage to kidneys (Ishioka, 1988). In patients with a mild renal dysfunction the single dose pharmacokinetics of TA was altered. The CL_s of TA was reduced by 50% while its V_d, did not change significantly in renally impaired subjects resulting in the prolongation of $t_{1/2}$ of the drug (Nilsen *et al.*, 1985). Jamali *et al.* (1988 and 1989) have studied the pharmacokinetics of TA in patients with renal dysfunction. The plasma concentration was initially low perhaps due to displacement of TA enantiomers from the albumin binding site resulting in the redistribution of the drug into the tissue compartment. On the other hand, there was an apparent reduction in the urinary excretion of conjugated TA enantiomers with the corresponding prolongation of $t_{1/2}$ for the intact drug due to *in vivo* hydrolysis of the acylglucuronide conjugates.

Murray *et a.l* (1992) assessed the effects of TA on renal function in patients with moderate renal insufficiency given single and chronic doses of TA. A reduction in inulin and creatinine clearance was small and reversible within 3 hours of dosing and a reduction of sodium was limited to the first two days after dosing. A lack of a chronic effect on glomerular filtration rate was also observed. In contrast, mild weight gain and elevated blood pressure and serum potassium concentrations occurred which may be clinically important in some cases.

Although drug absorption may be delayed in migraine attacks (Volans 1978), Pini et al (1990) assessed the pharmacokinetics of TA during and between migraine attacks and reported no differences in pharmacokinetic parameters between the two experimental situations.

Hosie and Hosie (1988) assessed the single and multiple dose pharmacokinetics of TA in elderly arthritic patients (age: 69 to 78 years). Renal clearance (CL_r) was decreased significantly in the elderly. The authors did not distinguish between the parent drug and its conjugated metabolites. There is insufficient data on the CL_r of acyl glucuronide conjugates of TA and its metabolites. Since ester conjugates of 2-APA are susceptible to enzymatic and nonenzymatic hydrolysis, a reduction in the CL_r of these conjugates may be clinically important. Nevertheless $t_{1/2}$ of TA was not different in elderly patients as compared to that observed in younger subjects. Additionally, the disposition kinetics of TA did not appear to change on multiple dosing and accumulation was not evident in a 1-week treatment period.

In pediatric patients the clearance of TA was 57% higher as compared to that of adults. The urinary excretion of the intact TA accounted for 33% of the dose. Furthermore, 48% of the dose was recovered as acyl glucuronide conjugates (Benarrosh *et al.*, 1989). This is significantly different from adults where the acylglucuronide conjugate of TA is the primary metabolite recovered in the urine (Jamali *et al.*, 1984).

1.4.3.5. Drug interactions

There is sparse information regarding the effects of other drugs on the PK of TA. Concomitant administration of aspirin has no effect on the pharmacokinetics of TA showing no major influence on bioavailability, mean transit time (MRT) and serum levels in man (Lucker et al 1982). This may be relevant because of the potential for self-administration of aspirin by patients already receiving NSAIDs. No significant PK interaction took place with concomitant administration of glibenclamide (10 mg) and TA daily in a rheumatoid arthritic patient with maturity onset diabetes (Daymond and Herbert 1982). Pentoxyphylline and phenprocoumon did not appear to affect the bioavailability of TA or its MRT (Lucker *et al.*, 1982).

Additionally antacids are sometimes co-administered with NSAIDs to counteract the adverse gastrointestinal side-effects. Lucker *et al.* (1982) studied the effect of commonly used aluminum hydroxide on the pharmacokinetics of TA. The co-administration of antacids had no effect on the rate or extent of absorption, and MRT of single dose TA in young volunteers.

Tiaprofenic acid does not appear to affect the rate of its own metabolism (no self induction) when administered over prolonged periods (Pottier *et al.*, 1982). Concomitant TA administration (200 mg three times daily) had no demonstrable effect on serum digoxin levels in healthy volunteers. (Doering and Isbary, 1983). The effects of TA on the plasma level of sulfamethizole were investigated in rats (Chiba *et al.*, 1987). Plasma levels of sulfamethizole were prolonged by co-administation of TA, and the clearance ratio was decreased after infusion which was attributed to competitive interactions between sulfamethizole and TA at the renal secretory level.

In hospitalized patients under anticoagulant therapy TA interacted with acenocoumorol. Increase in prothrombin time was greater in older patients and seemed to persist after discontinuation of the drug (Meurice, 1982).

1.4.4. Animal pharmacokinetics

Pottier *et al.* (1977) evaluated the pharmacokinetics of radioactively labeled TA in rats, mice, rabbits, and dogs. TA was rapidly absorbed from the GI tract. The absolute oral bioavailability of TA based on total radioactivity was 70-75% in rats and 100% in dogs.

In rats, the tissue distribution of TA was very limited, in general, the tissue concentrations of total radioactivity were lower than those observed in the plasma. The V_d of TA was determined to be 17% of body weight in the rat.

At least five different oxidative and reductive metabolites of TA were identified in the biological samples obtained from rats, mice, rabbits, and dogs. These metabolites account for only 2-5% of total radioactivity. Both TA and its metabolites are extensively conjugated with glucuronic acid and excreted into the urine (greater than 50% of administered dose). Clearance of TA was 27 mL/h/kg and 96 mL/h/kg in dogs and rats, respectively. Following administration of radioactive TA, a significant amount of radioactivity was recovered in the feces perhaps indicating extensive biliary excretion of TA and its metabolites. Indeed, in bile duct cannulated rats, approximately 70% of total radioactivity was recovered in the bile (Pottier *et al.*, 1977).

To date all studies done in animals have ignored the chiral nature of the TA molecule, hence, the assessment of disposition kinetics of R- and S-TA in animals is deemed essential.

1.4.5. Toxicity

Warrington *et al.* (1988) compared the GI blood loss in healthy male volunteers after regular (300 mg twice daily) and sustained release (600 mg once daily) formulations of TA and indomethacin using ^{51}Cr labeled erythrocytes. The blood loss was significantly lower after TA formulations as compared to indomethacin. Although the observed blood loss was apparently lower after the sustained release than regular release formulations of TA, this difference did not reach statistical significance (Warrington *et al.*, 1988). In a recent study the ulcerogenicity of several NSAIDs including TA was evaluated. Out of five patients on long term TA for treatment of rheumatoid arthritis, one developed an ulcer (Taha *et al.*, 1994). After multiple doses of aspirin, ibuprofen, and TA for 2 weeks, blood loss was significantly higher after aspirin than that observed after both TA and ibuprofen (Sorkin *et al.*, 1985). In rats, the dose at which 100% of animals developed an ulcer was 47 mg/kg for TA. The effects of TA on the GI mucosa was studied in normal volunteers and patients and compared to those observed after aspirin, ibuprofen and indomethacin. The GI damage was measured by assessing fecal blood loss, direct visualization employing endoscopy and measuring the transmural gastric potential difference. In general, the number and severity of gastric and intestinal lesions was significantly lower for TA as compared to those observed after indomethacin and diclofenac (Deraedt *et al.*, 1982).

Although the GI toxicity is the most common side-effect associated with TA, other complications such as phototoxicity and cystitis have also been reported. Following *in vivo* photo-activation, TA undergoes chemical degradation producing free radicals and/or singlet oxygens causing phototoxic reactions such as erythema, flaring, and urticarial weal (Bosca *et al.*, 1992). Phototoxicity of seven NSAIDs was studied in 31 patients with rheumatoid and osteoarthritis. Approximately 22% of patients (2 out of 9) who were taking TA experienced adverse reactions such as erythema and flaring, together with an urticarial response in four of six patients (Diffey *et al.*, 1983).

In one male and six female patients, long term TA use was implicated in the development of severe chronic cystitis (O'Neill 1994). Greene *et al.* (1994) have reported cystitis in 10 patients who were taking TA (300 mg twice daily) for at least 15 months whose conditions markedly improved after cessation of TA.

There is also one case report which implicates TA in causing liver dysfunction in a 56-year old female with polyarthropathy who was taking TA for 6 years and was on no other medications. The biochemical evaluation of the patient indicated abnormal levels of alkaline phosphates, alanine transaminase, γ-glutamyltranspeptidase which all returned to normal levels upon discontinuation of TA (Dankert *et al.*, 1986).

Delirium, as a rare toxic reaction of TA may also occur in elderly subjects. For example, an 89 year-old female patient suffering from osteoarthritis became delirious within 48 h after taking 200 mg of TA (Allison *et al.*, 1987).

Fetal exposure to TA in the first trimester of pregnancy did not result in any malformation or developmental problems, however, 25% of pregnant women exposed had miscarriages (Pastuszak *et al.*, 1993)

Tiaprofenic acid has been generally well-tolerated. Although the main observed side effects are related to the GI tract, it has a safer GI side effect profile as compared to other NSAIDs (Warrington *et al.*, 1988). Other side effects such as urinary tract complications or photosensitivity are infrequent and are not clinically serious problems.

1.5. Formulation-dependent gastrointestinal toxicity of nonsteroidal antiinflammatory drugs (NSAIDs)

1.5.1 Gastroduodenal side-effects

The design of new NSAIDs or re-formulation of existing ones is a current activity of pharmaceutical industry. The reasons for such interest in more NSAIDs is not to improve their efficacy but primarily to reduce their toxicity. For example, NSAID use is commonly associated with upper GI tract complications, including gastric and duodenal ulceration (Meddings, *et al.*, 1993). Several epidemiological studies in rheumatoid patients suggest up to 20% increase in the risk of developing a gastric ulcer with more variability in duodenal ulceration (Collins *et al.*, 1987 and Farah *et al.*, 1986). Cohort and case-control studies indicate even a higher risk of gastric bleeding associated with NSAID intake due perhaps to the small number of subjects included in these studies (Laporte et al., 1991). There is, however, the possibility of overestimation of the risk in case-control studies. For example, a number of large surveys have indicated that the relative risk of gastroduodenal complication is approximately 1-1.5% (Carson *et al.*, 1987 and Husby *et al.*, 1986).

The mechanisms by which NSAIDs induce gastric damage are multiple. After oral administration, NSAIDs (weak acids) accumulate in gastric mucosa due to the low pH of the stomach, as demonstrated for acetylsalicylic acid (ASA). The bicarbonate in mucus barrier increases the pH of the surface of the gastric cells to about neutral values resulting in a relatively high local concentration of ionized ASA at the surface of the cells. The next step involves passive diffusion of drug into the epithelial cells producing high local concentrations locally within epithelial cells. This provokes the uncoupling of oxidative phosphorylation and other ATPase-related processes leading to a change in mucosal permeability and even cell death. Prostaglandins (PGs) are endogenous compounds with a wide range of physiological activities including the regulation of mucosal defensive mechanisms. Bicarbonate secretion, mucus synthesis and subsequent secretion is reduced by NSAIDs because of the inhibition of PG production. It has also been suggested (Shorrock and Rees, 1989) that NSAIDs, particularly ASA, may disrupt the tight junctions between adjacent enterocytes and thus increase mucosal permeability to H^+ ions and/or impair the natural restitution ability of the gastric mucosa to repair itself. In addition, in recent years the importance of NSAID-induced disturbances in the mucosal microcirculation has become apparent. Reduced blood flow to the mucosa is an important factor in the pathogenesis of NSAID-induced gastroduodenal ulceration (Shorrock and Rees, 1989).

The asymptomatic nature of NSAID-induced gastroenteropathies renders diagnosis difficult (Meddings, *et al.*, 1993). In addition, endoscopically proven mucosal damage and patient complaints exhibit poor correlation (Aabakken *et al.*, 1990). An increase in the GI permeability prior to mucosal inflammation and the more serious complications such as ulceration have been reported. Thus, measurement of changes in the mucosal permeability of the GI tract may be used to predict and assess NSAID-induced GI toxicity.

1.5.1.1. Sucrose as a marker for NSAID induced gastroduodenal damage

Non-invasive methods using appropriate markers may be used to evaluate gastrointestinal permeability (Meddings, *et al.*, 1993 and Sutherland *et al.*, 1994). Sucrose has been suggested to be a suitable marker for the NSAID-induced upper GI damage (Meddings, *et al.*, 1993). Sucrose is a disaccharide and does not undergo significant absorption from the GI tract. After the ingestion of a noxious agent such as an NSAID, the gastric mucosal permeability to intact sucrose increases. Since sucrose is rapidly hydrolyzed to glucose and fructose in the small intestine, increase in the permeability of sucrose and its urinary excretion can be used as a surrogate measure of NSAID-induced upper GI damage (Meddings, *et al.*, 1993). Sutherland *et al.* (1994) have demonstrated a good correlation between severity of NSAID-induced upper GI damage and endoscopic findings. Patients with severe gastritis and gastric ulcer exhibited a significant increase in urinary excretion of sucrose compared to control group patients who did not receive NSAIDs.

In both an animal model (rabbits) and humans, sucrose permeability has proven to be a sensitive and noninvasive technique for screening upper GI toxicity of NSAIDs (Meddings, *et al.*, 1993 and Sutherland *et al.*, 1994). Recently Davies *et al.*(1995) have reported a new rapid, precise and sensitive method for the simultaneous determination of glucose directly and sucrose indirectly after hydrolysis of this disaccharide to its respective mono-sugars. Since in rats NSAIDs cause a pattern of change in the permeability of sucrose similar to that of humans, the rat model may be used to investigate the pathogenesis of NSAID-induced upper GI toxicity (Davies *et al.*, 1995).

1.5.2. Lower intestinal side-effects

It has been demonstrated that deleterious effects induced by NSAIDs are not confined to the gastroduodenal mucosa but may involve both the large and small intestine (Bjarnason *et al.*, 1993a). The presence of active inflammation in the small intestine of patients suffering from rheumatoid and osteoarthritis (n=97) and inflammatory bowel disease (n=26) have been evaluated using the indium-111 erythrocyte method. Indeed, asymptomatic enteropathy in 60-70% of patients on long-term therapy with NSAIDs has been observed (Bjarnason *et al.*, 1989). NSAID-induced distal intestinal inflammation may persist even 16 month after discontinuation of the drug (Bjarnason *et al.*, 1987). Both ^{111}In and ^{51}Cr-labelled studies indicate a significant blood and protein loss in patients with NSAID-induced intestinal inflammation. This may partially explain the observed anemia and hypoalbuminemia in patients with rheumatoid arthritis. Interestingly, it seems that the main site of bleeding in the GI tract is not the stomach but irather the small intestine (Bjarnason *et al.*, 1987, 1993b,1988 and Moris *et al.*,1992). Moreover, several case-studies implicate NSAIDs in causing intestinal strictures and lesions with multiple thin diaphragm-like septa which results in narrowing of the intestinal lumen (Bjarnason *et al.*, 1987 and Fellows *et al.*, 1992). Halter *et al.* (1993) have also reported ulceration and/or stricture of the ascending colon in patients who were taking sustained release diclofenac. In patients on long-term NSAID therapy (6 months or longer) intestinal permeability increased due to a disruption of the intestinal barrier function. Bjarnason (1989) has suggested that the observed increase in intestinal permeability results from the exposure of the mucosa to luminal macromolecules overwhelming the immune system. Subsequently, bacterial infiltration combined with the effect of NSAIDs on the neutrophil and normal intestinal repair processes, result in enteropathisies such as inflammation. It has been suggested that the intestinal inflammation caused by NSAIDs is due to the ability of these drugs to inhibit PG synthesis. This, coupled with the subsequent increase in leukotrienes which are associated with pro-inflammation, vasoconstriction, and production of free oxygen radicals may also trigger the disruption of the intestinal barrier function. In addition to PG/leukotriene imbalance, NSAIDs may affect the lysosomal membrane due to free oxygen radical production. Furthermore, NSAIDs may impair the oxidative phosphorylation cascade because of inhibition of glycolysis and the tricarboxylic acid cycle (Bjarnason *et al.*, 1989).

1.5.2.1. ^{51}Cr-EDTA as a marker for NSAID-induced intestinal damage

Disruption of epithelial integrity and changes in the permeability of the small intestine following ingestion of NSAIDs can be assessed using ^{51}Cr-EDTA as a marker (Bjarnason *et al.*, 1984). Urinary excretion of ^{51}Cr-EDTA has proven to be a reliable, sensitive, simple, and non invasive method for measuring intestinal permeability (Bjarnason *et al.*, 1984).

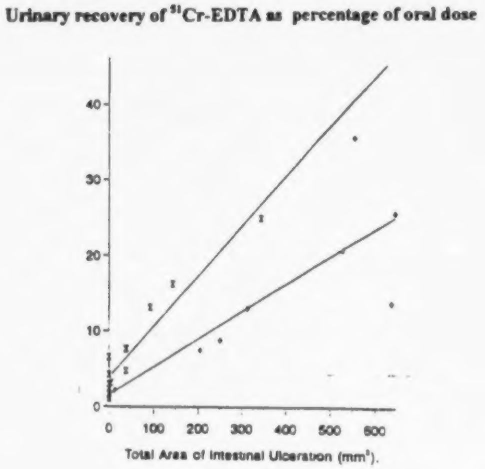

Figure 1.7. Graph of intestinal permeability (urinary recovery of ^{51}Cr-EDTA as percentage of oral dose) against area of intestinal ulceration (mm^2) subcutaneous piroxicam (x) or S(+)-ibuprofen (◆). Each point represents the data from 1 rat *(adapted from Ford et al., 1995).*

There are numerous reports indicating altered intestinal permeability by NSAIDs in humans. These studies, however, have been performed in the absence of any pharmacokinetic consideration and, thus, neither the time course of these changes nor their relationship to drug concentration have been established. After the subcutaneous administration of S(+)-ibuprofen and piroxicam, Ford *et al.* (1995) have demonstrated correlation between intestinal permeability and intestinal ulceration (Fig. 1.7). This indicates the sensitivity of intestinal permeability measurement to prediction of end-point ulceration which may develop after administration of NSAIDs.

Davies *et al*. (1994) have developed a rat model and studied the NSAID-induced small intestinal toxicity. Following administration of 10 μCi of ^{51}Cr-EDTA and 0-24 urinary collection, the base-line intestinal permeability was determined in Sprague-Dawley rats. The frequency distribution histogram of base-line intestinal permeability values exhibited a positive skew with a mean of 2.36±1.13% of dose excreted in urine. Administration of sulindac, nambumetone, naproxen, diclofenac, ibuprofen, flurbiprofen, indomethacin, and corticosterone resulted in an increase in the intestinal permeability. These changes were dose-dependent. Among the NSAIDs reported indomethacin caused the highest intestinal permeability changes which were partially reduced by concomitant administration of sulfasalazine, glucose/citrate, and misoprostol. It seems NSAID-induced intestinal permeability changes in rats follows a pattern similar to that observed in humans indicating the suitability of the rat as an animal model for studying the intestinal damage of NSAIDs (Davies *et al*., 1994).

NSAIDs may exert their detrimental effects on the GI tract either presystemically during absorption, or systemically following entry into the blood circulation, and inhibition of cyclo-oxygenase, consequently depriving the GI mucosa from cyto-protective prostaglandins. Therefore, the kinetics of GI release and absorption of NSAIDs becomes crucial in both magnitude and site of GI toxicity.

There are numerous approaches to reducinf the GI toxicity of NSAIDs, for example, using gastroprotective agents such as PG_E analogues, H_2-receptor antagonists, anti-muscarinic (M_1) agents, H^+-K^+-pump inhibitors, sucralfate, colloidal bismuth, prodrugs of NSAIDs, and antacids. The toxicity and efficacy of the aforementioned agents involved in gastroprotective therapy is not completely established (Rainsford, 1990). Another approach to reduce or eliminate the upper GI ulceration and bleeding associated with ingestion of NSAIDs is the modification of the release properties of NSAIDs formulations in order to by-pass the direct exposure of the stomach and duodenum to the drug. This approach prompted investigators to develop new dosage forms of NSAIDs such as enteric-coated and sustained release formulations. A consequence of altering the NSAID delivery system is the exposure of the small intestine to a high concentrations of the drug. These efforts to protect the upper GI tract from the toxic effects of NSAIDs may result in transferring the damage to the more distal segments of the intestine. Indeed, sustained release formulation of indomethacin (Osmosin^R) has been implicated in causing perforation in the small intestine (Abakken and Osens, 1989).

It may be argued that intestinal damage is not a common occurrence and mostly is asymptomatic, therefore, it is justified to ameliorate the upper GI toxicity of NSAID regardless of the possibility of damage to the small bowel. However, long term NSAID therapy may result in a serious blood and protein loss. Furthermore, in some patients surgical intervention may be necessary for small intestine strictures. Allison *et al.*, (1992) examined GI tract of 713 patients post-mortem. Of these 249 were NSAID users. Nonspecific small intestine ulceration was 8.4% in NSAID users and 0.6% in non-users. In addition, it has been determined that three patients died as a result of NSAID-induced perforated nonspecific small intestinal ulcers (Allison *et al.*, 1992). A case control study of

268 patients also suggests NSAID users are twice as likely to suffer from perforation or haemorrhage of small intestine (Langman *et al.*, 1985). Therefore, the risk/benefit of various formulations of NSAIDs with different release characteristics must be carefully evaluated by considering both the upper and lower GI tract.

1.6. Hypotheses

Based upon the foregoing background the following hypotheses were formulated:

1. Stereoselective release of 2-APA (TA and FB) from formulations containing one or more chiral excipients occurs.
2. Stereoselective release of TA enantiomers from certain dosage forms alters the pharmacokinetics of the enantiomers of TA in humans and rats.
3. The altered pharmacokinetics of individual enantiomers of TA due to the preferential input of enantiomers influences the GI toxicity of TA in rats.
4. There is a change in the site of TA-induced GI toxicity in rats following the administration of formulations with varying release rate.

1.7. Rationale and research plans

1.7.1. Reason for selection of 2-arylpropionic acids

NSAIDs, particularly, 2-APAs are amongst the most widely prescribed group of drugs worldwide. Every year in U.S.A. alone 100 million NSAID prescriptions are written (Bjarnason *et al.*, 1993a). The present trend in NSAID development is to alter the release properties of the NSAID dosage forms in order to formulate products with slow and/or site-specific release characteristics. The purpose of such modifications is to minimize the exposure of the gastroduodunenal mucosa to NSAIDs. This is a relatively n inexpensive and popular method among drug manufacturers in order to gain competative edge in the lucrative NSAID market.

The formulation and release rate-dependent GI toxicity of TA has not yet been studied. There is no information about the intestinal toxicity of TA. Investigating the TA-induced upper and lower GI tract toxicity may provide further understanding of mechanisms involved in the GI toxicity of NSAIDs and also elucidate on the importance of drug delivery systems on the extent and localization of mucosal damage caused by NSAIDs.

Formulation of a modified release product of NSAID requires addition of one or more specific types of excipients to the formulation may such excipients are obtained from natural sources, and therefore, are often chiral and optically pure (e.g. cellulose derivatives). To date, there are only two reports that indicate the possibility of stereoselective interaction and release of enantiomers from dosage forms (Duddu, *et al.*, 1993 and Aubry *et al.*, 1993). Therefore, there is a need to study this phenomenon for widely-used and clinically-important chiral drugs such as the 2-APA class of NSAIDs.

The mechanism by which chiral selectors like cyclodextrins discriminate between enantiomers involves the formation of hydrogen bonds between the hydroxyl groups, located at the entrance of the cyclodextrin cavity, and the drug enantiomers. Chiral recognition is dependent on additional factors such as tightness of fit between guest and host molecules, relative distance of the chiral centre of the guest molecule from the entrance hydroxyl groups, and the structure and size of the guest molecule. The presence of at least one aromatic ring in the racemic, guest molecule structure greatly increases the possibility of stereoselective interaction between the enantiomers and the cyclodextrin molecule (Armstrong *et al.*,1986). Since 2-APAs, including TA and flurbiprofen possess this structural feature, they are potential candidates for enantioselective interactions. Indeed, a stereoselective interaction of flurbiprofen enantiomers with TMCD has been reported (Imai *et al.*,1988).

The stereoselective release of enantiomers from the formulation may alter the PK of the individual enantiomers of TA. TA exhibits linear pharmacokinetics and lack of *in vivo* chiral inversion from R to S enantiomers in humans. Hence, due to absence of complicating factors, TA is a good candidate to study the effect of preferential input of enantiomers *in vivo*. Furthermore, the effect of stereoselective release of enantiomers and its potential effect on the GI toxicity of TA is an issue which needs to be further evaluated.

1.7.2. The development of a new direct chiral HPLC assay for quantification of TA in biological samples and validation of previously reported pre-column derivatization method

Using an indirect HPLC assay based on pre-column derivatization, the lack of stereoselectivity in the PK of TA has previously been reported. During the course of this study, however, a report suggested the possibility of the existence of a small but significant difference between PK of TA enantiomers. In order to validate previously reported, and our earlier PK data, a new direct stereospecific HPLC method was developed. Using both the direct and precolumn derivatization methods the PK of TA in humans has been studied.

1.7.3. Determine the possibility of stereoselective release from formulations containing chiral excipient(s)

A large number of chiral excipients are commonly included in the pharmaceutical formulations of chiral drugs. Therefore, the possibility of *in vitro* stereoselective release and dissolution of the selected 2-APAs were evaluated from formulations containing chiral excipients. The following formulations were either commercially available or they were prepared and evaluated in our laboratory:

- Commercially available sustained release beads
- DCD-TA inclusion complex
- HPMC-alginate system containing flurbiprofen
- Flurbiprofen entrapped in CM-chitin coated liposomes.

1.7.4. Determine the influence of stereoselective release on the pharmacokinetics of TA enantiomers

There is no significant stereoselectivity in the pharmacokinetics of TA in humans. A stereoselective change in pharmacokinetics of TA secondary to enantioselective alteration of the drug release from the formulation is, therefore, better detected using TA as the model drug. In order to investigate the effect of preferential release and their subsequent absorption, the pharmacokinetics of TA enantiomers were studied in humans following regular and sustained release formulations of TA.

The pharmacokinetics of TA enantiomers are also not known in the rat. Thus, the pharmacokinetics of TA after iv administration of the drug solution and oral administration as a powder, inclusion complex with DCD, and sustained release beads in male Sprague-Dawley rats were studied.

1.7.5. Investigate formulation-dependent GI toxicity of TA in rats

GI toxicity of NSAIDs may be formulation-dependent. Furthermore, a stereoselective change in the release, may alter the amount of the active enantiomer which comes in contact with the GI tract. Therefore, the gastroduodenal toxicity of racemic TA in formulations with different release characteristics [powder (regular release), inclusion complex with DCD, and a sustained release dosage form] was studied using sucrose. In addition, the time-course and the potential influence of formulation release kinetics on the toxicokinetics of TA, measured in terms of changes in the intestinal permeability (using ^{51}Cr-EDTA), following iv and oral administration of the above dosage forms were evaluated in rats. Furthermore, attempts were made to establish a meaningful relationship between drug concentration and effect observed after administration of various formulations.

2. EXPERIMENTAL

2.1 Chemicals

Racemic tiaprofenic acid (TA), and internal standard (IS) (±)-ketoralac [(±)-5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2-α]-pyrrole-1-carboxylic acid] tromethamine (KT), were obtained from Roussel Canada Inc., (Montreal, Canada) and Syntex Research (Palo Alto, CA), respectively. Flurbiprofen (FB), ketoprofen and naproxen (NAP) were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). 2,2,2-Trichloroethyl chloroformate and 4-dimethylaminopyridine (DMAP) were obtained from Aldrich (Milwaukee, WI, U.S.A.). L-Leucinamide hydrochloride, sucrose, DPPC, and Glucose-Trinders diagnostic kit were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). D-glucose was obtained from B.D.H. chemicals (Toronto, Canada). ELISA assay plates were obtained from Fisher Scientific (Edmonton, Canada). ^{51}Cr-EDTA (specific activity 570 MCi/mg) was purchased from Dupont NEN (Wilmington, U.S.A.). β-Cyclodextrin and DMCD were purchased from Sigma Chemical Company (St. Louis, U.S.A.) and used as supplied. The sustained release formulation (Surgam SR^R 300 mg capsules, lot# 0591 FGY) and the regular release tablets (Surgam^R 300 mg tablets, lot#0391 EJT) of TA were purchased from a retail pharmacy.

Isooctane, chloroform, HPLC grade water, H_2SO_4, NaOH, Na_2HPO_4, $MgCl_2$, and diethyl ether were purchased from B.D.H. chemicals (Edmonton, Canada). Methoxyflurane (Metofane^{TM}) was purchased from Pittman-Moore, Ltd. (Mississauga, Canada), polyethylene glycol 400 (PEG 400) from Fisher Scientific Ltd. (Edmonton, Canada) and heparin (Hepalean^{TM}) from Organon Teknika (Toronto, Canada). All other solvents and chemicals employed were of analytical reagent grade.

2.2. Indirect Stereospecific HPLC assay for TA

A previously reported stereospecific assay involving pre-column derivatization was used in this study (Mehvar *et al.*, 1988). Briefly, to 0.5 mL of plasma were added 0.05 mL of 100 mg/L solution of ketorolac internal standard (IS) and 0.1 mL of 0.6 M H_2SO_4. TA and IS were then extracted with 3 mL of isooctane:isopropanol (95:5), vortex mixed for 30 s and centrifuged at 1800 g for 5 min. The organic layer was transferred to a clean tube and evaporated to dryness. The residue was dissolved in 0.1 mL of 2 mg/mL solution of diethylaminopyridine (DMAP) in acetonitrile. The solution containing TA and IS enantiomers was derivatized with subsequent addition of 0.1 mL of trichloroethyl chloroformate (60 mM in acetonitrile) and L-leucinamide (1M in acetonitrile). After 2 min the reaction was stopped with 0.5 mL of 0.25 M HCL. The mixture was then extracted with chloroform. The chloroform layer was evaporated and the residue reconstituted in the mobile phase [0.06 M monopotassium phosphate:acetonitrile:TEA (70:30:0.02)]. Aliquots of 0.01 to 0.1 mL were injected on to a HPLC system (Waters, Mississauga, Canada) which consisted of a 590 pump, a variable wavelength 481 UV detector set at 310 nm, a 710B WISP autosampler, and a 10 cm x 4.6 mm analytical column with 5 μm octadecylsilane packing material (Partisil 5 ODS-3, Whatman, Clifton, NJ, USA). The recorder-integrator was a Hewlett-Pacakard model 3390A (Palo Alto, CA, U.S.A.). Mobile phase was pumped at a flow rate of 1 mL/min at ambient temperature. Under these conditions, the diastereomers of R- and S-TA were eluted at

17.2 and 20.5 min, respectively, while diastereomers of IS were eluted at 10.7 and 14.0 min (Fig. 3.1A). The linear range of the assay was 0.05 to 80 mg/L (Mehvar *et al.*, 1988). The minimum limit of quantification by this assay 0.05 mg/L with %CV values of 9.1% for R-TA and 8.3% for S-TA. Observed %Error values at 0.05 mg/L did not exceed 14.1% and 13% for R- and S-TA, respectively.

2.3. Direct Stereospecific HPLC assay for TA

The HPLC system was the same as that used for the indirect method. The resolution of TA enantiomers was achieved using a CHIRAL PACK AD column (CHIRAL TECHNOLOGIES INC., Exton, PA, USA) attached to a 5 cm Supelcosil LC-SI (Mississauga, Canada) column. Naproxen was used as IS Internal standard and both enantiomers of TA were detected at 310 nm. The mobile phase was hexane:isopropanol:trifluoroacetic acid (90:10:0.1) which was pumped at a flow rate of 1 mL/min at ambient temperature.

2.3.1. Standard solution

A stock solution of TA was prepared by dissolving 10 mg of the racemate in 100 mL of methanol. Blank plasma samples were spiked with varying volumes of TA stock solution to obtain final enantiomeric concentrations of 0.1, 0.5, 1, 2.5, 5, and 10 μg/mL. The IS solution was prepared by dissolving 10 mg of naproxen powder in 100 mL of methanol.

2.3.2. Sample preparation

To 0.5 mL of plasma samples containing various amounts of TA enantiomers were added 0.05 mL of IS solution and 0.1 mL of 0.6 M H_2SO_4. The enantiomers and IS were extracted with 3 mL of isooctane:isopropanol (95:5) followed by vortex-mixing for 30 s and subsequent centrifugation at 1800 g for 5 min. The clear organic layer was transferred to clean tubes and evaporated to dryness [Savant Speed Vac Concentrator-Evaporator (Emerston Instruments, Scarborough, Canada)]. The residue was reconstituted with 0.2 mL of mobile phase and aliquots of 0.01 to 0.1 mL were injected into the HPLC system.

2.3.3. Accuracy and precision

TA was added to plasma (n=5) to obtain concentrations of 0.1 μg/mL (low concentration) (n=5) and 5 μg/mL (high concentration) (n=5) of each individual enantiomer. The minimum quantifiable limit of the assay was found to be 0.025 μg /mL (n=3). The accuracy of the method was calculated based on the observed difference between measured and theoretical concentrations. Precision of the assay was evaluated using inter and intra-day coefficients of variation (CV%) in the response at the low and high concentrations.

2.3.4. Photostability of TA enantiomers

5 μg/mL solutions (5 mL) of TA enantiomers in pH 7.4 phosphate buffer were placed in sealed screw-capped glass vials under normal day-light conditions at ambient temperature for 110 h. Sample aliquots of 0.1 mL (n=3) were withdrawn at 0, 24, 48, 96, and 110 h. Samples were stored at -20°C until analysis by direct stereospecific HPLC assay.

2.3.5. Enantiomeric conversion Experiments

The possibility of enantiomeric conversion was studied under both direct and indirect HPLC assay conditionss. The S enantiomer of TA was enriched by the aliquot collection technique. An enriched aliquot containing S enantiomer as the major constituent was divided to two portions: 0.1 mL of the first portion was injected into the HPLC system to obtain enantiomeric composition of the mixture immediately after collection (direct method) (n=3). The second portion was evaporated to dryness and derivatized with 0.1mL of trichloroethyl chloroformate (60 mM in acetonitrile) and L-Leucinamide (1 M in acetonitrile) as described in the sample preparation section (n=3). In order to investigate the possibility of enantiomeric interconversion within 24 h, a small aliquot of the same solution, stored in amber glass inserts at room temperature, was injected into the HPLC system sequentially at 10, 30 min, and 24 h.

2.4. Indirect Stereospecific HPLC assay for Flurbiprofen

A previously reported stereospecific assay was used to quantify flurbiprofen enantiomers (Berry and Jamali, 1988). Briefly, to 0.5 mL of plasma were added 0.05 mL of 100 mg/L solution of ketoprofen (IS) and 0.2 mL of H_2SO_4 (0.6 M). Flurbiprofen and IS were then extracted with 3 mL of isooctane:isopropanol (95:5), vortex-mixed for 45 s and centrifuged at 3000 rpm on a Clay-Adams centrifuge for 5 min. The top layer was transferred to a clean tube and 3 mL of HPLC grade water was added. The mixture was again mixed vigorously and centrifuged for 5 min. The organic layer was aspirated and 0.35 mL of H_2SO_4 (0.6 M) and 3 mL of chloroform were added. Following centrifugation for 5 min, the aqueous layer was aspirated off and the chloroform phase was evaporated to dryness using a SVC 100H Savant Speed Vac Concentrator and Refrigerated Condensation Trap (Emerston Instruments, Canada). The residue was dissolved in 0.1 mL of 50 mM solution of TEA in acetonitrile. The solution containing flurbiprofen and IS enantiomers were derivatized by subsequent addition of 0.05 mL of ethyl chloroformate (60 mM in acetonitrile) and L-leucinamide (1M in acetonitrile). After 2 min the reaction was stopped with 0.05 mL of HPLC grade water. A 0.01 to 0.05 mL of aliquot of this mixture was used in analysis. The mobile phase consisted of 0.067 M monopotassium phosphate:acetonitrile:TEA (65:35:0.02). The Waters HPLC system (Waters, Mississauga, Canada) consisting of a Model 6000A pump, a 710B WISP auto-injector, and a 490 multiple wavelength UV detector was operated at ambient temperature. Flurbiprofen and IS were detected at 250 and 275 nm, receptively. The column was a 10 cm x 4.6 mm analytical column with 5 μm octadecylsilane packing material (Partisil 5 ODS-3, Whatman, Clifton, NJ, USA). The recorder-integrator was a Hewlett-Packard model 3390A (Palo Alto, CA, U.S.A.). The mobile phase was pumped at a flow rate of 1 mL/min at ambient temperature. Under these conditions, the diastereomers of R- and S-flurbiprofen were eluted at 17 and 21 min, respectively, while diastereomers of R and S of IS were eluted at 8 and 10 min, respectively. Minimum limit of quantification for each enantiomer was 0.1 mg/L (in 0.5 mL) (Berry and Jamali, 1988).

2.5. Measurement of radioactivity in the urine samples after administration of ^{51}Cr-EDTA

Urine samples were counted directly in a Beckman Gamma 8000 multisample counter (Beckman, Irvine, CA) for 1 minute over an energy range of 0-2 Mev. Two blank samples (1mL H_2SO_4 + 10 mL tap water) were counted with every set of urine samples. The relative permeability was calculated as a percentage by dividing the sum of cpm present in 24 h sample by the cpm of the dosing solution after correcting for background radiation. Changes in the intestinal permeability following TA administration po (powder, sustained release, and inclusion complex) and iv were expressed as percent increase in the 0-24 h urinary excretion of the ^{51}Cr-EDTA (Davies *et al.*, 1994).

2.6. Measurement of sucrose in urine samples

The amount of sucrose in urine was determined using a previously reported method (Davies *et al.*, 1995). Briefly, 0.2 mL urine sample divided into 100 μL aliquots were used for quantification of sucrose levels in urine. To one of the 0.100 mL aliquots of urine sample 0.025 mL of 2 M H_2SO_4 was added in order to hydrolyze sucrose to its monosaccharides. Then both 0.1 mL aliquots of urine sample were incubated for 10 min in a boiling water bath. The pH of the aliquot which was subjected to hydrolysis using acid was added 0.04 mL of 2 M NaOH. Samples were then diluted to 0.500 mL using Sorensen phosphate buffer (pH 7.4) followed by 1 mL Glucose Trinders (contains glucose oxidase and peroxidase). After vortex-mixing (30 s), samples were left at room temperature for 18 min. Finally, 0.05 mL of the solution was transferred to well-bottom ELISA plates and the absorbance was measured at 490 nm using an ELISA plate reader.

The reaction of glucose in the sample with the components of Glucose Trinders reagent results in the formation of a quinoneimine dye which is proportional to the original amount of glucose in the sample and can be quantified colorimetriclly.

2.7. Formulations containing chiral excipients

2.7.1. Preparation of matrix containing racemic flurbiprofen and HPMC-alginate system

Racemic flurbiprofen (200 mg) and HPMC (100 mg) were dispersed into an aqueous solution of sodium alginate (0.5% w/v) heated to 60°C. In some of the experiments DMCD (1080 mg) [flurbiprofen:DMCD (1:1 M ratio)] was also added. The sustained release beads were formed by dropping the dispersion through a disposable syringe onto gently agitated calcium chloride solution (Bodmeier *et al.*, 1991). The gelled beads were separated after 5 min by filtration, rinsed with distilled water, and oven-dried at 60°C for 12 h.

2.7.2. Preparation of CM-chitin-coated liposomes

Liposomes were prepared using the emulsion method reported by Dong and Rogers (1991). DPPC and flurbiprofen (2:1 molar ratio) in 3 mL of dichloromethane were combined with 1 mL of pH 7.0 phosphate buffer, then sonicated at 10 KHz for 4 min to form a w/o emulsion. The emulsion was diluted with 1 mL of CM-chitin aqueous solution (1% w/v), then vortex-mixed to form w/o/w emulsion. Subsequently, the organic solvent was slowly removed under reduced pressure by rotary evaporation resulting in CM-chitin coated liposomes (Dong and Rogers, 1991). Furthermore, pH of the aqueous phase was adjusted 2, 3.7, 4.2, 4.7, and 7.4 corresponding to 0, 25, 50, 75, 100 (%)

ionization in order to assess the effect of degree of ionization on the encapsulation efficiency and stereoselective interaction of the enantiomers of flurbiprofen (pK_a: 4.2).

2.7.3. Preparation of diethyl-ß-cyclodextrin inclusion complex

2.7.3.1 Synthesis of diethyl-ß-cyclodextrin

Heptakis-(2,6-di-O-ethyl)-ß-cyclodextrin was prepared by modification of a previously described method (Szejtli *et al.*, 1980). ß-Cyclodextrin was dissolved in a 1:1 mixture of DMSO:DMF; $Ba(OH)_2.8H_2O$ and BaO were then added in portions with continuous stirring over a period of 20 min. After cooling the mixture to 0°C, diethyl sulfate was slowly added with vigorous stirring over a period of 2 h. The temperature of the reaction mixture was kept below 10°C and stirring was continued for an additional 72 h. After reaction of excess diethyl sulfate with ammonium hydroxide solution, the mixture was extracted with ethyl acetate and the product crystallized on standing.

2.7.3.2. Structure confirmation

Proton and carbon-13 NMR spectra of the DCD in DMSO-d_6, with tetramethylsilane as internal reference, were performed on a Bruker AM-300 FT NMR spectrometer at 22°C and 75°C. The FAB mass spectrum were recorded in nitrobenzyl alcohol as well as glycerin on an MS9 A.E.I (Manchester, England) spectrometer. Operating conditions: Mass range 132-1750, sampling rate 256, signal level threshold 1, minimum peak width 5, scan rate (sec/dec) 10.0, total scan in run 7.

2.7.3.3. Preparation of DCD inclusion complex

The inclusion complex of TA and DCD was prepared by the kneading method (Yasuhide *et al.*, 1990). Tiaprofenic acid (150 mg) and DCD (900 mg) (1:1 molar ratio) were triturated with 2-5 mL of water. The slurry was thoroughly kneaded for an additional 40 min and the resulting material was then freeze-dried (Freeze Dryer 4.5, Labconco Corp. Kansas City, MI). Formation of the inclusion complex was evaluated and confirmed by differential scanning calorimetry (model2910 TA Instruments Inc., Newcastle, DE) at a heating rate of 10^{o}C/min under nitrogen. The samples were stored at -20^{o}C until analysis.

2.8. *In vitro* release and dissolution studies

2.8.1. Release of flurbiprofen enantiomers from liposomes coated with CM-chitin

The release of flurbiprofen enantiomers from the liposomes coated with CM-chitin was studied using dialysis bags with a molecular weight cut-off point of 12000 Dalton (Amicon, Beverly, U.S.A.). The dialysis bags filled with 3-5 mL of the suspension of liposomal pellets were then incubated in a 900 mL release medium (phosphate buffer pH 7.4) which was previously equilibrated to 37±0.5 °C. The release medium was agitated at 50 rpm during the period of the experiment. Samples were withdrawn from the release medium at 0, 0.5, 1, 2, 3, 5, 8, 12, 24 h. The samples were stored at -20^{o}C until analysis.

2.8.2. Release of flurbiprofen enantiomers from HPMC-alginate and HPMC-alginate-DMCD systems

The release of flurbiprofen enantiomers from HPMC-alginate and HPMC-alginate-DMCD beads was studied using a USP basket apparatus (USP XXI). The dissolution medium was 900 mL phosphate buffer (pH 7.4) and stirred at 50 rpm. The temperature of dissolution medium was adjusted at 37±0.5 °C. At scheduled intervals (0, 0.5, 1, 2, 3, 5, 8, 12, 24 h) 1 mL aliquots were withdrawn and filtered through a filter paper (Whatman filter paper No. 4). The samples were stored at -20°C until analysis.

2.8.3. Release and dissolution of TA enantiomers from regular release tablets and sustained release capsules

Dissolution studies were performed using a USP basket apparatus (USP XXI). Nine sustained release capsules and 3 regular tablets were tested. The dissolution media were phosphate buffer solutions pH 7.4 [for both regular release tablets (n=3) and sustained release capsules (n=9)] and 8.0 [for sustained release capsules (n=3)]. The volume of dissolution medium was 900 mL. The media were stirred at 50 rpm at 37.0±0.5°C and samples were withdrawn at 0, 0.25, 0.5, 1, 2, 3, 4, 5, 12, 24 h. For the sustained release formulation an additional sample was also collected at 8 h. The samples were stored at -20°C until analysis.

2.8.4. Release and dissolution of TA enantiomers from the regular release powder and DCD inclusion complex

The dissolution of TA as a powder and as its inclusion complex with DCD (<100 mesh) was studied at three different pH values; pH 1.5 (KCl-HCl buffer, ionic strength, μ =0.4), 3 (Sorensen Glycine-HCl buffer, μ=0.2), and 7.4 (Sorensen phosphate buffer, μ =0.27). On separate occasions, 10 mg of rac-TA powder and its inclusion complex (equivalent to 10 mg of powder) were dispersed in 900 mL of dissolution medium previously equilibrated at $37^{o}\pm0.5^{o}$C. The dissolution medium was stirred at 50 rpm and samples were withdrawn from the dissolution medium using a syringe attached to a filter [Millipore (pore size: 0.22 μm) Millipore Corporation (Bedford, Massachusetts)], samples were taken just prior to addition of the products, and at 0.08, 0.17, 0.25, 0.33, 1.0, 2.0, 3.0, 6.0, 8.0, 12.0, and 24.0 h after the addition of the products. The samples were stored at -20^{o}C until analysis. Six release studies were performed at pH 3 for the inclusion complex while the rest of the dissolution experiments were repeated in triplicate.

2.8.4.1. Solubility studies

The solubility of TA was determined in the presence and absence of DCD. An excess amount of racemic TA was added to 1 mL of buffer solution at pH 3 (Sorensen Glycine-HCl buffer, μ=0.2) and 7.4 (Sorensen phosphate buffer, μ=0.27). The same experiment was repeated in the presence of 100 mg of DCD (equivalent to amount of DCD used in dissolution experiments). Samples (0.01 mL) were taken after 24 h and 48 h. All experiments were performed in triplicate at 37^{o}C.

2.9. *In vivo* studies in humans

2.9.1. Effect of stereoselective release on pharmacokinetics of TA enantiomers

Four healthy male volunteers (average age 27.8±5.0 years and body weight of 86.3±11.8 kg) participated in this study. The study was conducted according to the Declaration of Helsinki. Following overnight fasting, subjects received single 300 mg doses of TA in sustained release capsules and the regular tablets in a cross-over fashion. Food was not allowed 3 h post-dose. There was a one week washout period between the two treatments. No other medication or alcohol containing beverages were allowed during the period of the study. Venous blood samples were collected via an indwelling catheter at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 24 h. All specimens were stored in acid-washed containers at -20°C until analysis.

2.9.2. Validation of previously reported pharmacokinetic studies

In order to examine and confirm previously reported results describing the PK of TA enantiomers, the above experiment was repeated after recruitment of new subjects but using the same study protocol a year later. The study was conducted according to the Declaration of Helsinki. Four healthy male volunteers (average age 37.3±11.2 years and body weight of 86.5±14.3 kg) participated in this study.

2.10. *In vivo* studies in rats

2.10.1. Pharmacokinetics of tiaprofenic enantiomers in rats

A total of 35 male Sprague-Dawley rats with body weight of 300-350 g were used in this study. All rats were catheterized by insertion of silastic tubing (0.025 inch i.d x 0.047 inch o.d.) into the right jugular vein. Animals were fasted overnight with free access to water. Rats received 20 mg/kg single iv dose of TA in PEG 400 via inserted cannula (n=10), and 20 mg/kg single oral dose of TA as powder, inclusion complex or sustained release beads (n=5). Oral formulations were administered as solid particles via gastric tubing. Food (a standard rat chow) was available 2 h post-dose. Blood samples (0.2 mL) were withdrawn from the jugular vein cannula at 0, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 24 h post-dose. After administration of SR formulation, an additional blood sample was collected at 12 h post-dose. All specimens were kept at -20°C until analysis.

2.10.2 Toxicokinetics of tiaprofenic acids in rats

2.10.2.1 TA-induced intestinal damage

The lower GI damage caused by the different formulations of TA was assessed using Sprague-Dawley rats (weight: 300-350 g). During the period of experimentation animals were housed at ambient temperature and humidity in individual metabolic cages with a wire mesh floor allowing complete urine collection. Animals were fasted overnight with free access to water. Food (a standard rat chow) was given 2 h post-dose. In order to examine the base-line intestinal permeability, 0.5 mL of an aqueous solution containing 10 μCi/mL of ^{51}Cr-EDTA was administered orally using an 18 gauge 5 cm curved feeding needle (Havard Apparatus) attached to a 1 mL syringe. After a 24 h washout period, 20 mg/kg single oral dose of TA as inclusion complex (n=5), sustained release (n=5), or regular release powder (n=5) were administered. TA was also given as iv bolus_via inserted jugular vein cannula (n=5). Since the extent of absorption was not complete after the administration of inclusion complex (F; 25%) and SR (F; 28%), TA dose for regular release powder and iv bolus doses were adjusted to produce the same systemic availabilities as those observed after oral modified release dosage forms (F; 25%). After administration of the different dosage forms, 0.5 mL of ^{51}Cr-EDTA was given orally to different groups (5 rats per group) at 1, 2, 3, 4, 6, 8, 12, 24, 48 h. In order to establish whether the selected dose (20 mg/kg) is located in the linear ascending portion of the dose-response curve or not, on separate occasions 20 and 40 mg/kg single oral doses of inclusion complex (n=5) and powder (n=5) were administered, followed by ^{51}Cr-EDTA at 3 and 12 h post-dose. Following oral administration of DCD and physical mixture of DCD:TA (1:1 molar ratio, equivalent to the amount present in a single dose of inclusion complex), the distal intestinal permeability was measured using ^{51}Cr-EDTA given at 3 h post-dose. The latter experiment was performed to evaluate the contribution of DCD on the overall observed intestinal toxicity. Based on PD-time profiles, the maximum increase in the intestinal permeability occurred 3 h post-dose. Therefore, ^{51}Cr-EDTA was administered 3 h post-dose to assess the intestinal damage caused by DCD and the physical mixture. Following ^{51}Cr-EDTA administration, urine was collected 0 to 24 h in cups containing 1 mL of H_2SO_4 (1M) to inhibit microbial growth. At each collection 10 mL of tap water was used to rinse the urine collection tray. The urine was transferred to capped glass scintillation vials and immediately counted.

2.10.2.2 TA-induced upper GI damage

Upper GI damage by TA following administration of regular release powder, inclusion complex, or sustained release formulations was assessed using a total of 9 Sprague-Dawley rats (weight: 300-350 g). To test base-line permeability, a 1.0 mL solution containing 1g/mL of sucrose was administered orally to each individual rat. The damage caused to the upper GI tract by 20 mg/kg single oral dose of TA given as powder (n=3), sustained release (n=3), or inclusion complex (n=3) was studied. The dose of TA given as powder was adjusted to produce the same systemic availability as that observed after inclusion complex or sustained release formulations (F:25%). One hour after administration of TA as powder, inclusion complex, or sustained release dosage forms, 1 mL sucrose solution (1.5 g/mL) was given to rats followed by 0-24 h urinary output collection.

2.11. Data analysis

2.11.1. Pharmacokinetic and pharmacodynamic data

Following iv and oral (powder, inclusion complex, and sustained release) administration of TA, the plasma concentration of the drug was plotted *vs* time. The pharmacokinetic model best describing time-courses of the enantiomers and total drug (R-TA + S-TA) was determined using PCNONLIN version 4.1 (Metzler, 1969). The elimination rate constant (β) were estimated from the terminal portion of the fitted curves. For fitting purposes, the concentration time data were not weighted. The area under the plasma TA concentration-time curves from 0-24 h (AUC_{0-24}) was calculated using the linear trapezoidal rule. The total area under the plasma TA concentration-time curve ($AUC_{0-\infty}$) was the sum of AUC_{0-24} and extrapolated area (C^*/β), where C^* was the last concentration quantified. The systemic clearance after iv administration was estimated as $CL = Dose/AUC_{0-\infty}$. The apparent volume of distribution was calculated using $V_d = Dose/\beta \cdot AUC_{0-\infty}$. Peak plasma concentration (C_{max}) and time of its attainment (T_{max}) were determined from the experimental data points.

In order to obtain a meaningful relationship between concentration of TA [S-TA (active enantiomer) and total (R-TA + S-TA)] and intestinal permeability, a previously described conventional link PK-PD model proposed by Holford and Sheiner (1981) was used (Fig. 3.21A, Appendix 1). In addition, a modified version of the above link PK-PD model was developed with a direct input into the effect compartment (DINP) and used for analysis of the relationship between the plasma concentrations of TA and the time course of intestinal (Fig. 3.21B), where K_a, K_{1e}, K_{eo}, and K_{10} are rate constants for absorption, transfer from central to effect compartment, equilibration between effect and concentration of the drug in the effect compartment, and elimination of the drug from central compartment, respectively. K_0 is a zero-order rate constant for the direct input of drug into the effect compartment.

A stepwise approach was taken for analysis of the underlying relationship between concentration of TA and intestinal toxicity using DINP model. Systemic CL, V_d, and K_{10} were estimated after iv administration. Absorption rate constant K_a and equilibration rate constant K_{eo} was estimated after administration of regular release powder. Assuming a small effect compartment with no discernible influence on the concentration of drug in the central compartment, K_{1e} was arbitrarily set to be 1000 times smaller than the estimated value for K_{eo} (Sheiner *et al.*, 1979). Considering rapid absorption of the drug in the upper GI tract after regular release powder, the direct exposure of the distal intestine to TA was assumed to be negligible. Therefore, the value of K_0 was set to be 0 after oral administration of TA as a regular release powder.

The direct input model (DINP) employed the following differential equations with numerical integration, to estimate concentration of TA in the effect compartment.

$$\frac{dX_E}{dt} = k_0 + k_{1e} X_c - k_{eo} X_e \ (2)$$

Derivation of integrated equations describing concentration-time profiles of the drug in the effect compartment and the relationship between this concentration with measured pharmacological/toxicological effect are depicted in Appendix 1.

The relationship between concentration of drug in effect compartment and the intestinal toxicity can best be described using a sigmoid E_{max} model, $E = (E_{max} \cdot C_e^{\gamma})/(C_e^{\gamma} + EC^{\gamma}_{50})$, where E_{max} is maximum effect, C_e is concentration of drug in the effect compartment estimated using conventional or DINP link PK-PD models, and γ is a number influencing the slope of the curve (Holford and Sheiner, 1981). The quality of fit was assessed using Akaike (1978) and Schwartz (1978) methods.

2.11.2. Statistical comparison among observations

Using the Student's t-test for paired data (α=0.05), the significance of the differences between the enantiomers release profiles in each and every dissolution medium was assessed. Also the significance of the differences observed between the two enantiomers by pooling the data points from all dissolution experiments was tested. The significance of difference between enantiomers was assessed using the paired Student's t-test while comparison between treatment groups was made using the unpaired t-test, at α=0.05. The difference between more than two means was evaluated using one-way

ANOVA followed by the Duncan's multiple range test. All results are expressed as means ± S.D..

3. RESULTS

3.1 Validation of the direct stereospecific assay

Under the chromatographic conditions used in this method, the two enantiomers of TA and IS eluted as sharp peaks at 13.0, 14.8 and 11.1 min, respectively (Fig. 3.1b). There was no interfering peak with either TA or IS enantiomers in the blank plasma. Due to unavailability of optically pure individual enantiomers of TA, the order of elution for R- and S-TA could not be determined directly. It has been reported, however, that derivatization of 2-APA with L-leucinamide (Fig. 3.1A) results in elution of R enantiomer prior to its antipode (Mehvar *et al.*, 1988). Following derivatization of enriched aliquot of S-TA according to the method described by Mehvar (1988) and injection into the HPLC system (indirect method), the retention time of the main component in the aliquot corresponded to the retention time of the second peak. In addition, in rats S-TA is the predominant enantiomer (Muller *et al.*, 1993). Similarly, in rats it was observed that the predominant enantiomer was eluted earlier than its antipode indicating the same order of elution for TA enantiomers under the chromatographic conditions of both direct and indirect methods. Excellent linearity was observed between the peak-area ratios (R- and S-TA/IS) and corresponding plasma concentrations (0.025 to 10 μg/mL) with $r^2>0.998$. Typical linear equations describing standard calibration curves were $Y=0.191X+0.002$ for R-TA and $Y=0.187X+0.002$ for S-TA.

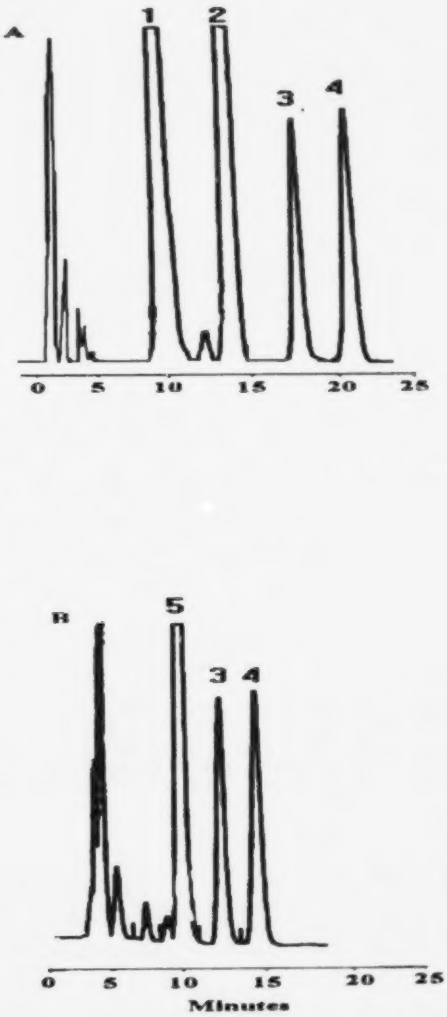

Figure 3.1. Chromatogram of 100 ng/mL of TA in plasma using indirect (A) and direct (B) methods [key: 1 and 2, R and S ketorolac (IS); 3 and 4, R- and S-TA; 5, S-naproxen (IS)].

Since both direct and derivatization (Mehvar et al., 1988) methods used the same extraction solvent [isooctane:isopropanol (95:5)], it was not necessary to evaluate the extraction efficiency (>95%) for the direct method. The percent difference between added and measured concentration values (% error) were smaller than 10.6% for R-TA and 9.9% for S-TA at low concentration (0.1 μg/mL). The calculated % error values at high concentration (5 μg/mL) were 7.96% and 8.02% for R- and S-TA, respectively. The method is reproducible as reflected by the inter- and intra-day CV% values for the peak-area ratios at low and high concentrations and slopes of the standard calibration curves (Table 3.1). The lowest examined plasma concentration, 0.025 μg/mL, had a CV% of 9.7% and 8.0% for peak-area ratios of R- and S-TA, respectively. The % error between intended concentration (0.025 μg/mL) and measured concentration did not exceed 8.5% for R-TA and 11.4% for S-TA.

Table 1.3. Precision of the direct HPLC method for TA enantiomers (n=3).

	0.1 (mg/L)		**5 (mg/L)**		**Slope**	
	R	**S**	**R**	**S**	**R**	**S**
Intra-day CV%	8.31	7.41	6.19	6.50	3.49	3.45
Inter-day CV%	8.58	8.43	6.95	6.88	2.99	3.25

3.1.1. Photostability

Under fluorescent lighting, concentrations of TA enantiomers were slowly decreased with degradation $T_{50\%}$ values of 13.6±1.9 and 12.7±0.13 days for R and S enantiomers, respectively (n=3) (Fig. 3.2). Photo-degradation reactions followed first-order kinetics as reflected by a log linear decline in enantiomer concentrations during the period of the study.

3.1.2. Enantiomeric conversion experiments

Reanalysis of a highly-enriched aliquot of S-TA via direct stereospecific assay indicated the presence of 1.43±0.19% R-TA as optical impurity. There was no change in the level of optical impurity of enriched aliquot after 24 h under the chromatographic conditions described for the direct stereospecific HPLC method that was developed. On the other hand, a significant increase in the concentration of R-TA was observed when S-TA was subjected to derivatization with L-leucinamide. Following correction for the original optical impurity in the sample, a 6.95±3.06% (n=3, ranging from 4% to 10%) conversion to R-TA was noticed during the sample preparation under the conditions described for the indirect method.

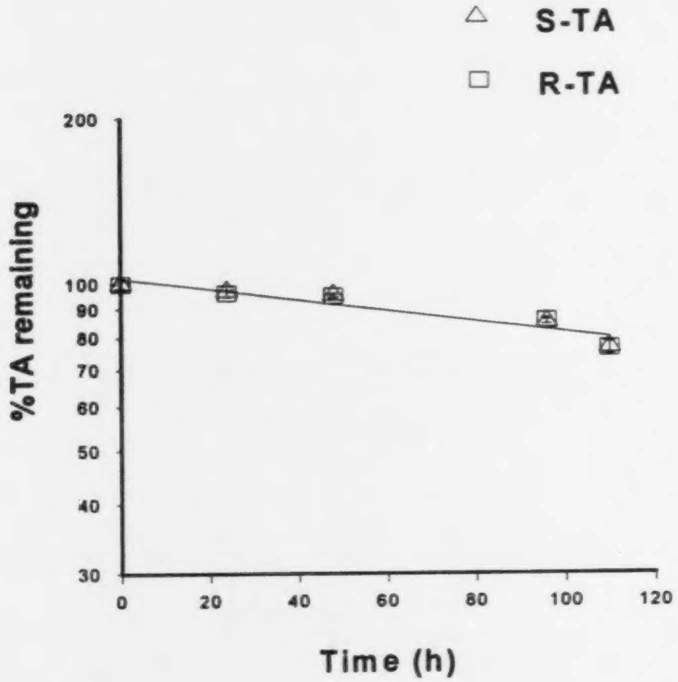

Figure 3.2. Photo-degradation of TA enantiomers in buffer solution (5 µg/mL) in normal laboratory illumination.

3.2. Structural confirmation of diethyl-β-cyclodextrin

NMR spectral data of the DCD are depicted in figures 3.3A and B and corresponding assignments given in Tables 3.2. and 3.3. The results indicate that the ß-cyclodextrin starting material was ethylated at the 2- and 6- hydroxyl positions. Although the product may be a mixture of ethylated cyclodextrins, with different degrees of substitution, it is particularly apparent from the carbon NMR spectrum that 2,6-diethyl-ß-cyclodextrin is the principal component. The proton and carbon spectra were comparable to the 400 MHZ spectra kindly provided and recently published (Hirayama *et al.*, 1993).

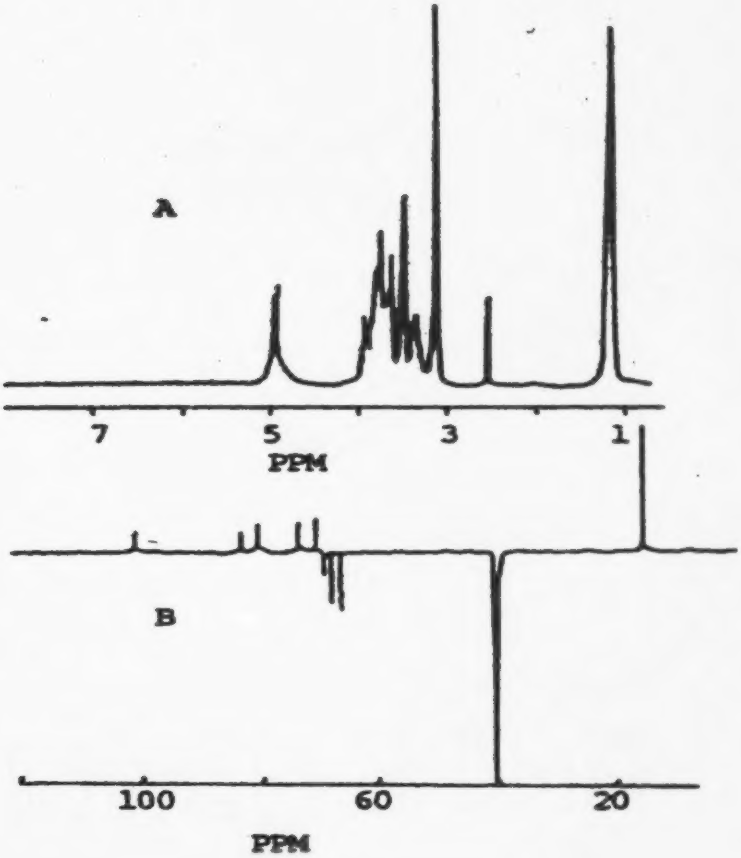

Figure 3.3. ^{1}H-NMR spectrum of diethyl-β-cyclodextrin obtained in DMSO-d_6 at 75°C (A), ^{13}C-NMR spectrum of diethyl-β-cyclodextrin obtained in DMSO-d_6 at 75°C (B)

The FAB mass spectrum obtained in the nitrobenzyl alcohol matrix gave a pseudo-molecular ion with a m/z ratio of 1630.75 corresponding to a molecular formula of $C_{70}H_{126}O_{35}$[2Na.NaCl]. When glycerin was employed as a matrix, a pseudo-molecular ion with m/z ratio of 1711.90 corresponding to $C_{70}H_{126}O_{35}[2C_3H_8O_3]$ was observed.

3.3. Confirmation of the formation of inclusion complex between TA and DCD

Figure 3.4. represents the DSC curves of freeze-dried racemic TA, DCD, a 1:1 molar ratio of TA:DCD mixture prepared by simple physical mixing, and the product obtained by kneading (inclusion complex). The disappearance of the endothermic peak at approximately 92°C in the DSC pattern of the inclusion complex may indicate inclusion complexation of rac-TA and DCD. However, we were not able to obtain the exact stoicheometry of the interaction between enantiomers of TA and DCD due to the lack of solubility of the inclusion complex.

3.4. In vitro release and dissolution studies

3.4.1. Release of flurbiprofen from liposomes coated with CM-chitin

The release and subsequent dissolution of FB enantiomers from CM-chitin coated liposomes were not stereoselective (Fig. 3.5. and 3.6.). Adjusting the pH of the aqueous phase during preparation of liposomes failed to alter the pattern of stereoselectivity in the release profiles of FB enantiomers (Fig. 3.5. and 3.6.). However, the extent of encapsulation was significantly affected by the pH of the buffer used in the preparation of liposomal formulations (Fig. 3.7.). At pH values equal to or lower than the pK_a (4.22) of the drug the exent of encapsulation was ≥80% while increasing the pH of the buffer above the value of the pKa of FB resulted in a significant reduction in the extent of encapsulation (≤60%) ($p<0.05$)

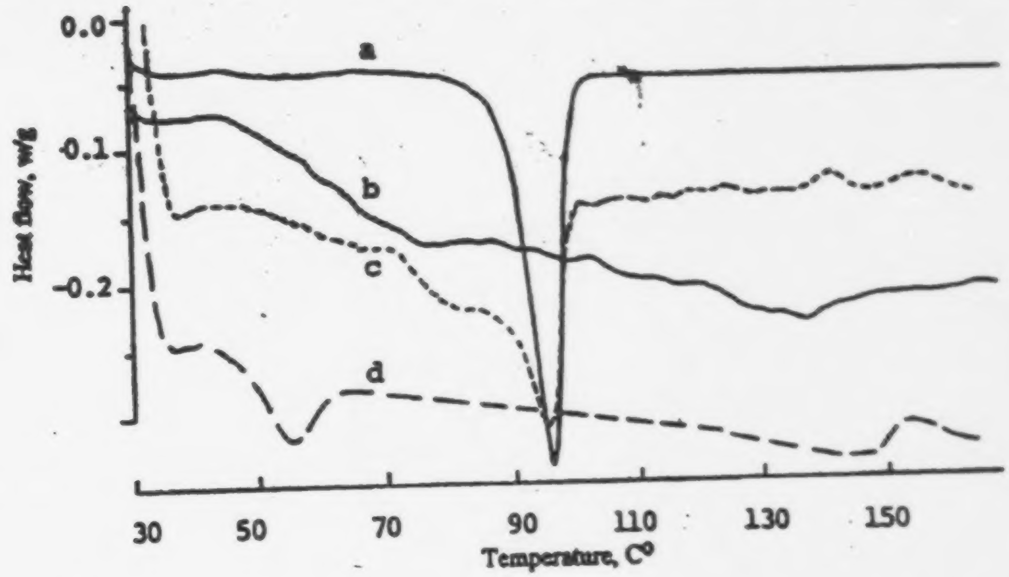

Figure 3.4. DSC curves of freeze-dried tiaprofenic acid (a), freeze dried diethyl-β-cyclodextrin (b), freeze-dried physical mixture (c), and freeze-dried inclusion complex (d).

3.4.2. Release of Flurbiprofen from HPMC-alginate and HPMC-alginate-DMCD systems

The release and dissolution of FB enantiomers from HPMC alginate system were slow and nonstereoselctive with a lag time of 2 h for both R- and S-FB (Fig. 3.8.A). Incorporation of DMCD into the formulation resulted in a faster release of enantiomers from the formulation with no lag time compared to that observed after HPMC-alginate beads. The lack of stereoselectivity was also observed in the release profiles of the enantiomers from the formulation containing DMCD (Fig. 3.8.B).

3.4.3. Release and dissolution of TA enantiomers from regular release tablets and sustained release capsules

Figure 3.9A depicts a representative dissolution profile of the regular release formulation at pH 7.4. The release of the enantiomers was rapid and showed no stereoselectivity. In the first 30 min of dissolution, an average of 78.50% of R enantiomer and 77.30% of S enantiomer were dissolved. The difference between the release enantiomers was not statistically significant. The release of R- and S-TA from the sustained release capsule was slower than that observed after regular release tablets. At pH 7.4, the release was incomplete (within 24 h, 66.9% and 60.7% R and S enantiomers were dissolved, respectively) and apparently stereoselective (Fig. 3.9B): The concentration of the R enantiomer was consistently higher in all dissolution experiments. Seven of 9 dissolution tests exhibited a statistically significant difference between R and S enantiomers throughout the experiment ($p<0.01$-0.04). Furthermore, when data points from all experiments were pooled, the difference was highly significant ($p<0.0001$). The difference between 24 h cumulative percent release ranged from 1.5% to 29% with a mean of 10.1±9.3%, indicating a large inter-product variation in stereoselectivity.

At pH 8.0, after 24 h, the release from the sustained release capsules was almost complete (90.93% and 89.73% for R- and S-TA, respectively) with no significant difference between the release profiles of the enantiomers (Fig. 3.9C).

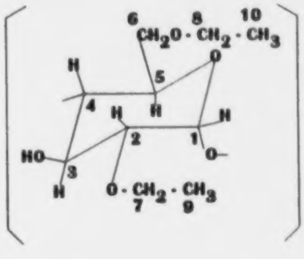

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Table 3.2. Assignments for 300 MHZ proton NMR of heptakis-(2,6-di-ethyl)-β-cyclodextrin in DMSO-d_6.

Chemical Shift (ppm from TMS)	Relative Number of Protons	Assignment
4.92	1	C3 OH
4.88	1	C1 H
3.90	1	C7 H
3.80	1	C3 H
3.75	1	C5 H
3.70	1	C7 H
3.68	1	C6 H
3.61	1	C6 H
3.50	2	C8 H
3.80	1	C4 H
3.31	1	C2 H
1.0-1.25	6	CH_3

Table 3.3. 300 MHZ ^{13}C NMR of heptakis-(2,6-di-ethyl)-β-cyclodextrin in DMSO-d_6.

Chemical Shift (ppm from TMS)	Assignments
100.70	C1
82.90	C4
79.90	C2
72.80	C3
69.90	C5
68.70	C6
67.20	C7
65.20	C8
14.97	C9, C10

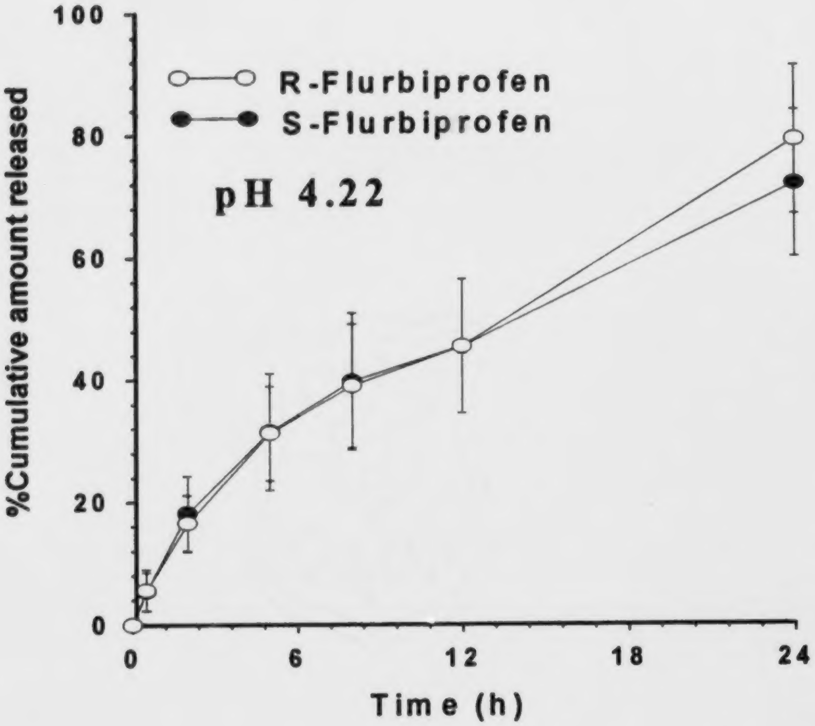

Figure 3.5. Release of flurbiprofen from CM-chitin coated liposomes

[aqueous pH adjusted to 4.22 (50% ionization)]

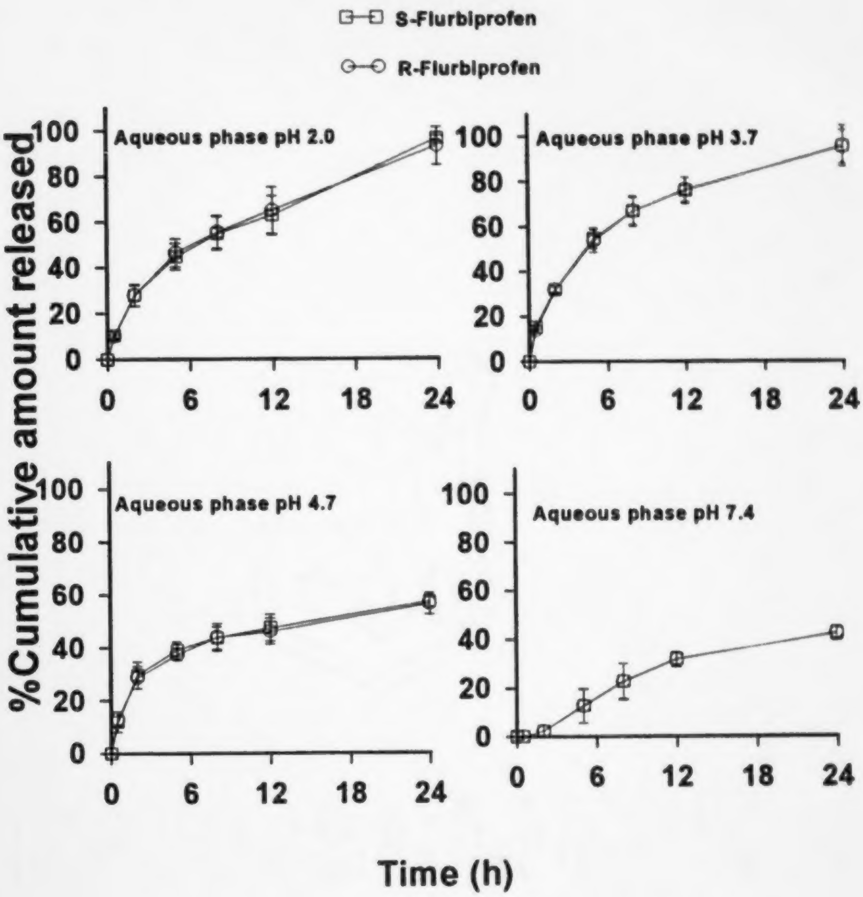

Figure 3.6. %Cumulative release of flurbiprofen from various CM-chitin DPPC liposomal formulations

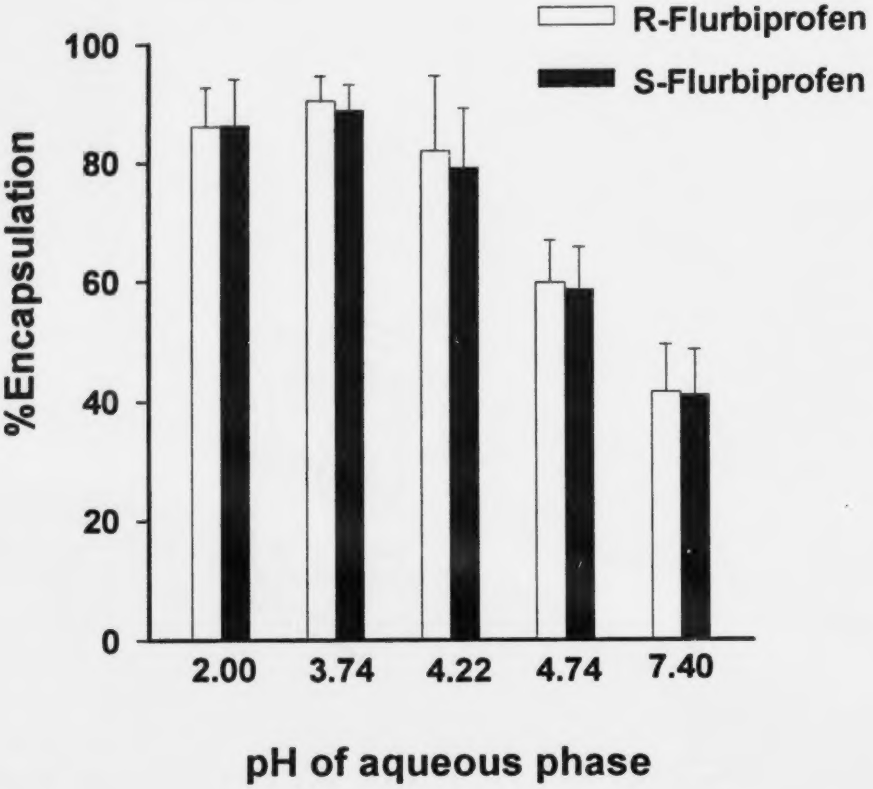

Figure 3.7. The effect of pH of aqueous phase used in the preparation of CM-chitin coated liposomes on their encapsulation efficiency

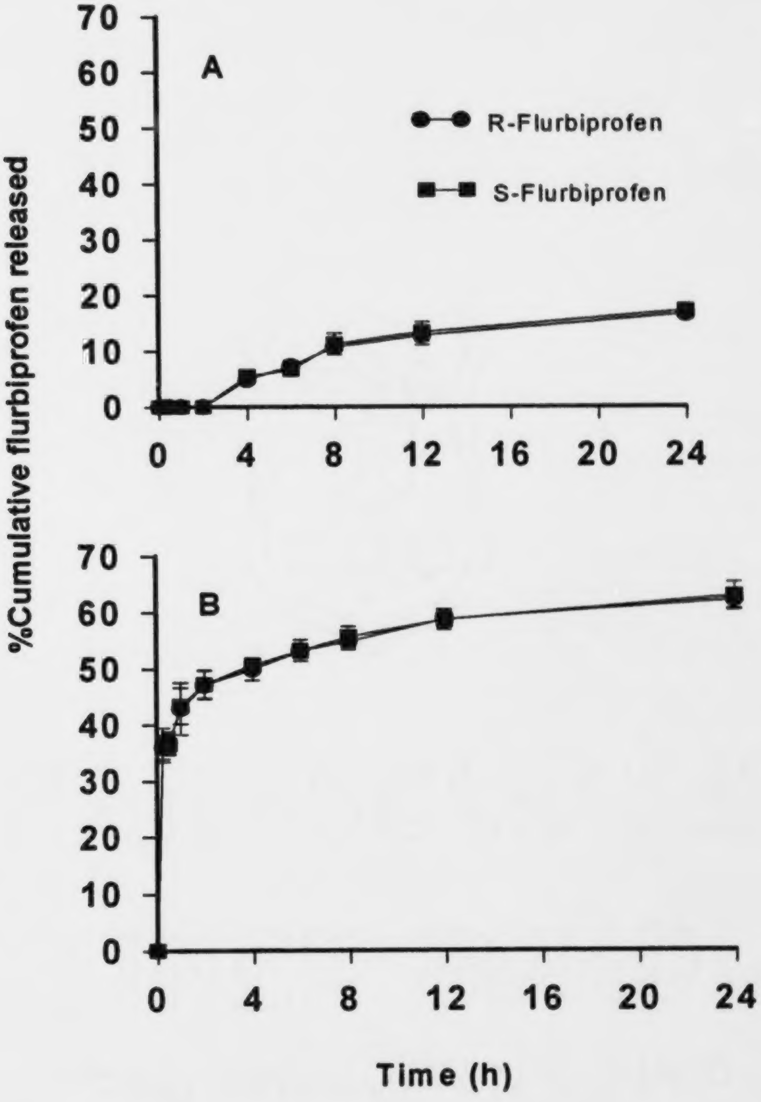

Figure 3.8. %cumulative release of flurbiprofen from HPMC-alginate beads (A), and HPMC-alginate-DMCD beads (B)

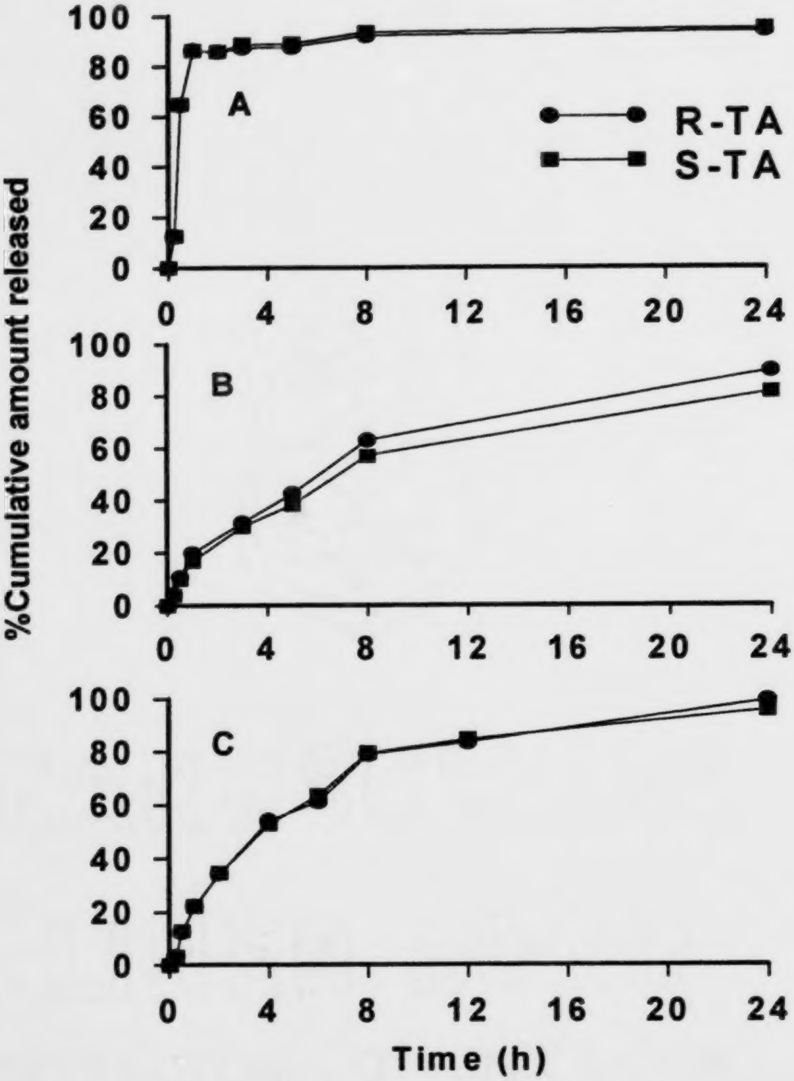

Figure 3.9. Representative plots of cumulative percentage of R- and S-tiaprofenic acid release *vs* time from a regular release tablet at pH 7.4 (A), and from a sustained release capsule at pH 7.4 (B), and 8.0 (C)

3.4.4. Release and dissolution of TA enantiomers from regular release powder and DCD inclusion complex

A representative dissolution profile of the TA enantiomers, when TA was added to the pH 1.5 dissolution medium as a powder, is depicted in Fig. 3.10A. A lag-time of 0.22±0.03 h was observed for both enantiomers and their dissolution profiles were superimposable. Within 24 h of the experiment, 20.52±3.01% of R-TA and 20.47±2.83% of S-TA were dissolved [S:R % cumulative released within 24h (ΣR24) ratio: 0.99±0.07]. Significant stereoselectivity in the dissolution of the enantiomers was not observed.

The concentrations of the enantiomers released from the inclusion complex at pH 1.5 could not be measured as they were below the sensitivity limit of our assay.

Dissolution profiles for TA enantiomers from TA powder at pH 3.0 are illustrated in Fig. 3.10B; dissolution was not stereoselective. Compared to pH 1.5, the ΣR24 values were higher and there was no statistically significant difference between R and S enantiomer concentrations. The slopes of the dissolution rate plots were not different between enantiomers (the release rate constants, K_R: 0.56±0.0479 h^{-1} and 0.553±0.0471 h^{-1} for R-TA and S-TA, respectively). The ΣR24 values of 54.40±14.01% and 55.9±11.76% were observed for R- and S-TA, respectively, with an S:R ΣR24 ratio of 1.04±0.05. Furthermore, dissolution of the enantiomers from the powder did not exhibit any lag-time.

At pH 3.0, the release of TA enantiomers from the inclusion complex and their subsequent dissolution, were significantly slower than observed with the powder (Fig.

3.11A) (K_R: 0.169±0.0458 h^{-1} for R-TA and 0.174±0.0464 h^{-1} for S-TA). A significant stereoselectivity was found between release profiles of R- and S-TA in every experiment performed at this pH ($p<0.05$). Moreover, when data points from all experiments were pooled, the difference was also found to be significant ($p < 0.05$). In general, the release of R-TA was faster than that of S-TA. There was a trend towards a shorter lag-time (0.50±0.77 h for R-TA *vs* 0.92±1.28 h for the antipode) and greater ΣR24 for the R enantiomer [12.80±8.83% and 11.11±7.60% for R- and S-TA, respectively (S:R ratio: 0.88±0.04)].

The dissolutions of the enantiomers from powder at pH 7.4 were rapid (K_R: 3.28±0.238 $h^{-1}$and 3.28±0.222 h^{-1} for R- and S-TA, respectively) with ΣR24 of 92.87±4.38 and 94.16±5.85% for R- and S-TA, respectively (S:R ratio: 1.04±0.02). There was no stereoselectivity in the dissolution patterns of the TA enantiomers as indicated by the superimposable dissolution profiles (Fig 3.10C).

At pH 7.4, the release of R- and S-TA from the inclusion complex was considerably faster compared to that observed at pH 1.5 and 3.0 (K_R: 2.49±0.251 $h^{-1}$for R-TA and 2.50±0.252 h^{-1}). Both enantiomers were immediately released without any lag-time. As shown in Fig. 3.11B, the release profiles of the enantiomers were similar and there was an appreciable reduction in stereoselectivity. At this pH only one of three release experiments gave evidence of significant stereoselectivity ($p=0.0034$). After 24 h, 49.28±4.46% of R-TA and 47.98±5.86% of S-TA were released (S:R ratio: 1.01±0.03). This difference between ΣR24 values was not statistically significant.

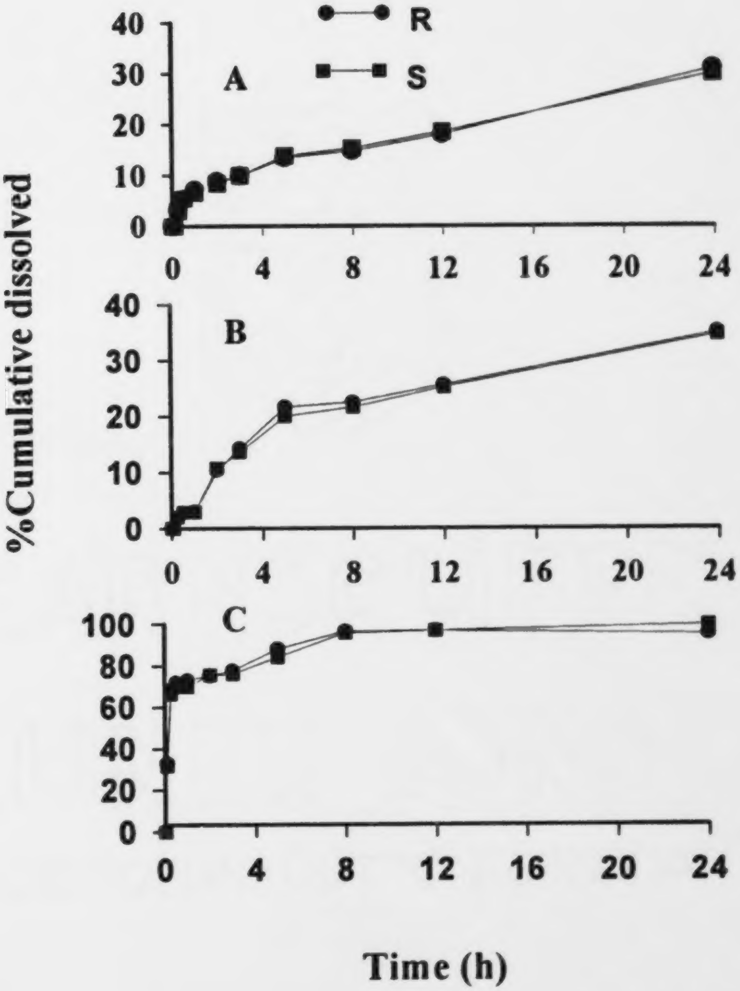

Figure 3.10. Representative plots of cumulative percentage of dissolved R- and S-tiaprofenic acid powder *vs.* time at pH 1.5 (A), 3.0 (B), and 7.4 (C)

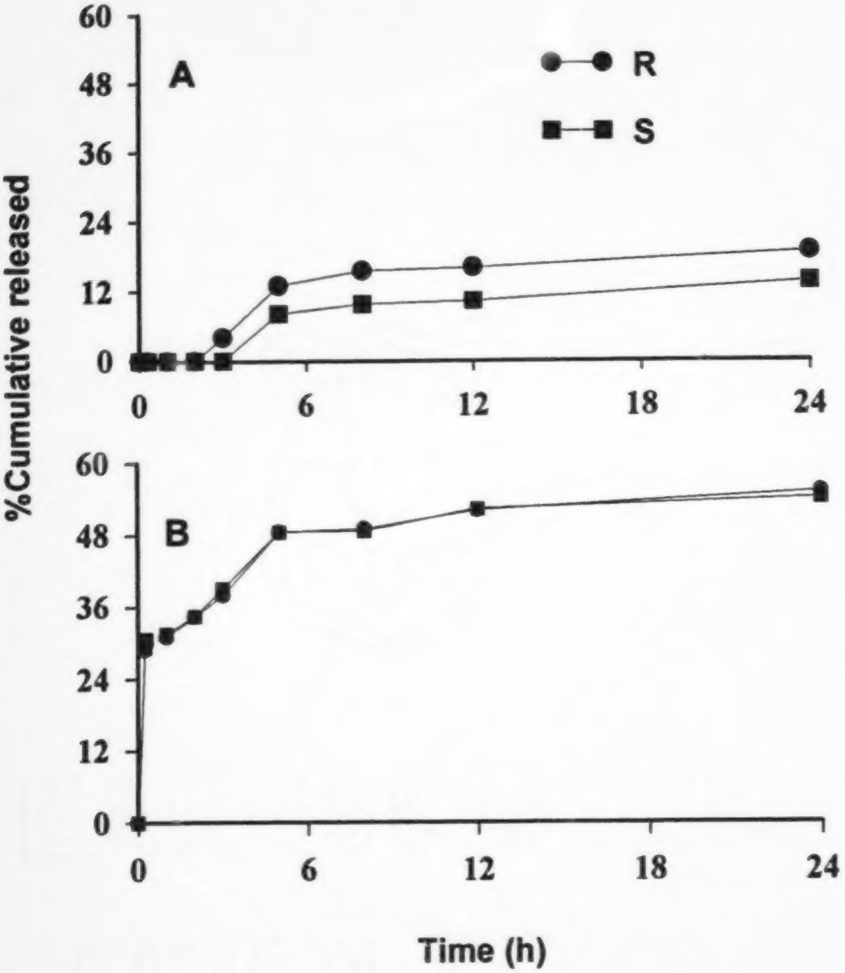

Figure 3.11. Representative plots of cumulative percentage of dissolved R- and S-tiaprofenic acid inclusion complex *vs* time at pH 3.0 (A) and 7.4 (B)

3.4.4.1. Solubility experiments

Equilibrium was established within 48 h, since the concentrations obtained at 72 h were not different from those observed at 48h. Solubilities of both enantiomers of TA were 0.0142 ± 0.0008 mM in pH 3 buffer at 37°C. Presence of DCD did not alter the solubilities of the enantiomers ($P > 0.05$).

Higher solubility values were obtained for TA enantiomers in pH 7.4 buffer at 37°C (0.38 ± 0.05 mM) with no stereoselectivity. Addition of DCD into the buffer system did not alter the solubility of the enantiomers at pH 7.4 ($p > 0.05$).

3.5. *In vivo* studies in humans

3.5.1. Effect of stereoselective release on the pharmacokinetics of TA enantiomers

The data following oral administration of the sustained release (Fig. 3.12B) and the regular release (Fig. 3.12A) formulations were both best described using first-order absorption kinetics with one- and two-compartment open models, respectively.

After the regular tablets, the plasma concentration of both TA enantiomers peaked at approximately 1.5 h (Fig. 3.12A, Table 3.4). The observed t_{max} after the sustained release formulation was 4.0 h for both enantiomers (Table 3.4). The apparent $t_{1/2}$ was longer after sustained release capsules compared to the regular formulation (Table 3.5). Similar to the regular tablets, after sustained release formulation, the plasma concentration profiles of the two enantiomers were not significantly different (Fig. 3.12B) (S:R $AUC_{0\text{-}12}$ ratio of 0.99 ± 0.02 after regular tablets and 1.02 ± 0.05 after sustained-release capsules).

Despite the observed significantly longer t_{max} and lower C_{max} and stereoselectivity in the release of enantiomers at pH 3.0 after the sustained-release capsules, there was no significant difference between the AUC values of the two formulations (Table 3.4).

After oral administration of both pharmaceutical dosage forms, approximately 70% of the dose was excreted in urine mainly as glucuronide conjugates (Table 3.4) and with a low amount of intact drug (<10% of dose). No significant stereoselectivity was observed in the cumulative urinary excretion of the enantiomers (conjugated and unchanged) in either treatment group [S:R 24 h cumulative urinary excretion ratio of 0.95±0.02 and 0.97±0.04 for regular-release tablets and sustained-release capsules, respectively].

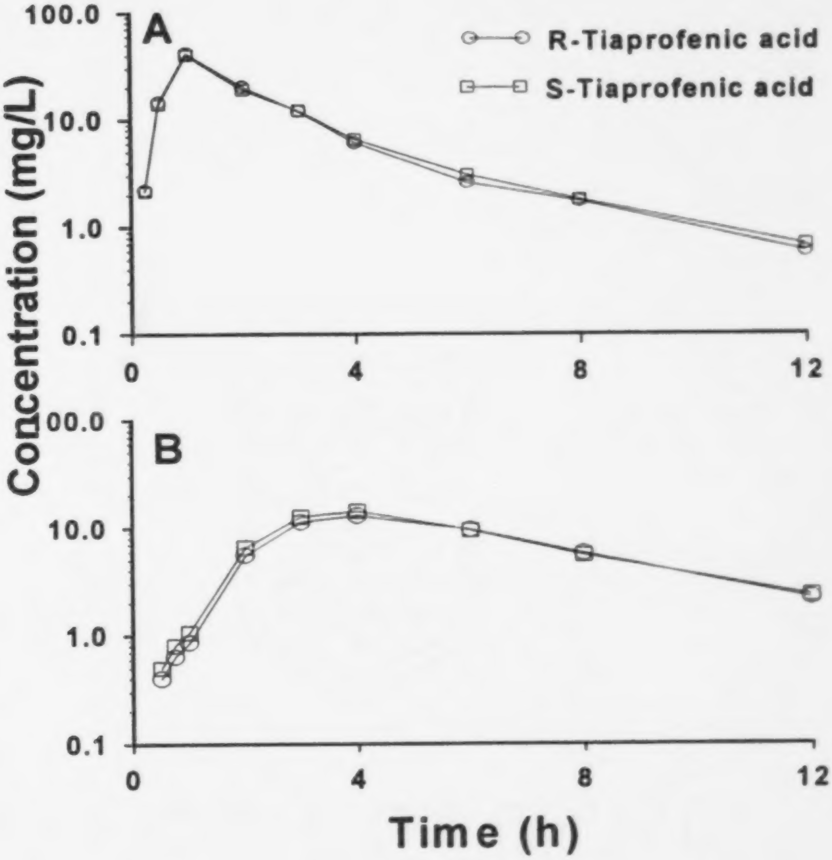

Figure 3.12. Representative (Vakily and Jamali, 1994) plasma concentration-time profiles of R- and S-TA following administration of regular-release tablets (A) and sustained-release capsules (B).

Table 3.4. Bioavailability parameters after oral administration of single 300 mg doses of racemate

	Lag time(h)		C_{max} (μg/mL)		C_{12}[a] (μg/mL)		AUC_{0-12} (μg.h/mL)		$\sum U_{m24}$[b] (% of dose)	
Subjects	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
					Regular-Release Formulation					
1	0.00	1.0	40.3	41.7	1.31	0.97	90.1	90.5	67.4	68.9
2	0.25	1.0	34.5	37.2	0.69	0.59	118.1	118.0	75.9	80.0
3	0.00	1.0	24.6	27.1	0.31	0.22	56.0	57.9	71.6	74.2
4	0.50	3.0	18.9	18.7	0.00	0.00	59.0	58.3	69.4	75.7
Mean	**0.19**	**1.5**	**29.6**	**31.2**	**0.58**	**0.45**	**80.8**	**81.2**	**71.1**	**74.6**
S.D.	**0.21**	**0.86**	**8.4**	**8.9**	**0.56**	**0.43**	**25.3**	**25.1**	**3.2**	**4**
					Sustained-Release Formulation					
1	0.25	4.0	14.3	13.0	2.9	2.2	82.2	78.1	69.1	73.6
2	0.25	4.0	20.3	18.7	2.3	2.1	123.7	113.9	81.5	79.5
3	0.25	4.0	10.2	12.3	2.3	2.2	63.5	67.0	64.6	69.2
4	2.00	4.0	7.1	7.1	1.3	1.2	32.8	32.8	82.7	75.1
Mean	**0.68**	**4.0**[d]	**13.0**[c]	**12.8**[c]	**2.2**[c]	**1.9**[c]	**75.5**	**72.9**	**72.0**	**74.4**
S.D.	**0.75**	**0.00**	**5.0**	**4.1**	**0.65**	**0.50**	**32.9**	**33.4**	**6.2**	**3.7**

a. Concentration at 12 h; b. Cumulative urinary excretion of acylglucuronated enantiomer in 24 h; c. Significant difference between products.
d. Significantly different from the corresponding value for -TA

Table 3.5. Pharmacokinetic indices following single 300 mg doses of regular release tiaprofenic acid.

	$t_{1/2}$ (h)		CL/F (mL/min)		V_d/F (L)	
Subject	*S*	*R*	*S*	*R*	*S*	*R*
1	2.8	2.8	26.9	27.2	6.5	6.5
2	2.7	2.6	20.3	20.5	4.8	4.7
3	2.1	1.8	44.0	42.7	7.8	6.8
4	1.1	1.2	42.4	42.9	4.1	4.3
Mean	2.2	2.1	33.4	33.3	5.8	5.6
S.D.	0.7	0.7	10.1	11.3	1.5	1.1

3.5.2. Validation of previously reported pharmacokinetic studies

In order to assess the validity of results obtained earlier in this study, on a separate occasion the pharmacokinetics of TA enantiomers were re-evaluated after administration of regular release tablets (300 mg) and sustained release capsules (300 mg). Plasma samples were analyzed using both the previously reported pre-column derivatization method and the recently developed direct stEreospecific HPLC assay.

The results obtained using two different analytical methods [(Fig. 3.13A, B, C, and D) and (Table 3.6 and 3.7)] were similar to those observed earlier [(Fig. 3.12A and B) and (Table 3.4 and 3.5)] in this study.

There was an excellent correlation between measured concentrations using the indirect method and those measured using the direct HPLC method (regular release: R-TA: r=0.9902, slope=1.07 and S-TA: r=0.9909, slope=0.98; SR: R-TA: r=0.9671, slope=0.93 and S-TA: r=0.9884, slope=1.10) (Fig. 3.14A, B, C, and D).

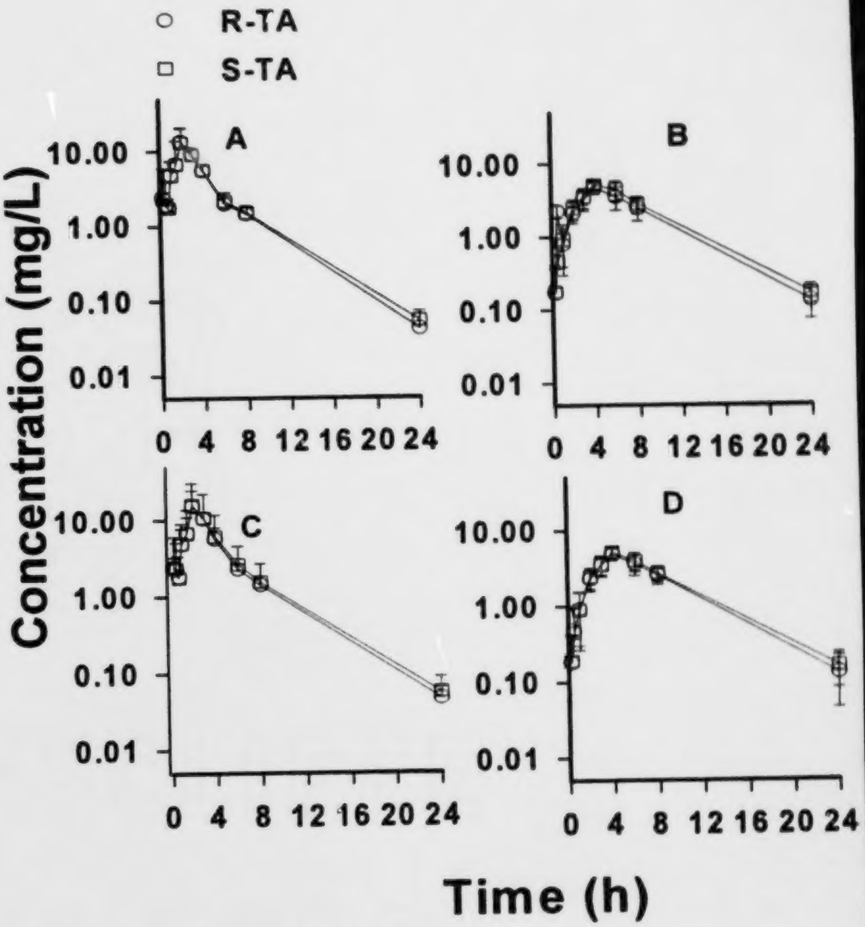

Figure 3.13. Mean plasma concentration-time course of TA enantiomers followi single oral dose of 300 mg as regular release tablets (A. Indirect method, C. Direct method) and sustained release capsules (B. Indir method, D. Direct method)

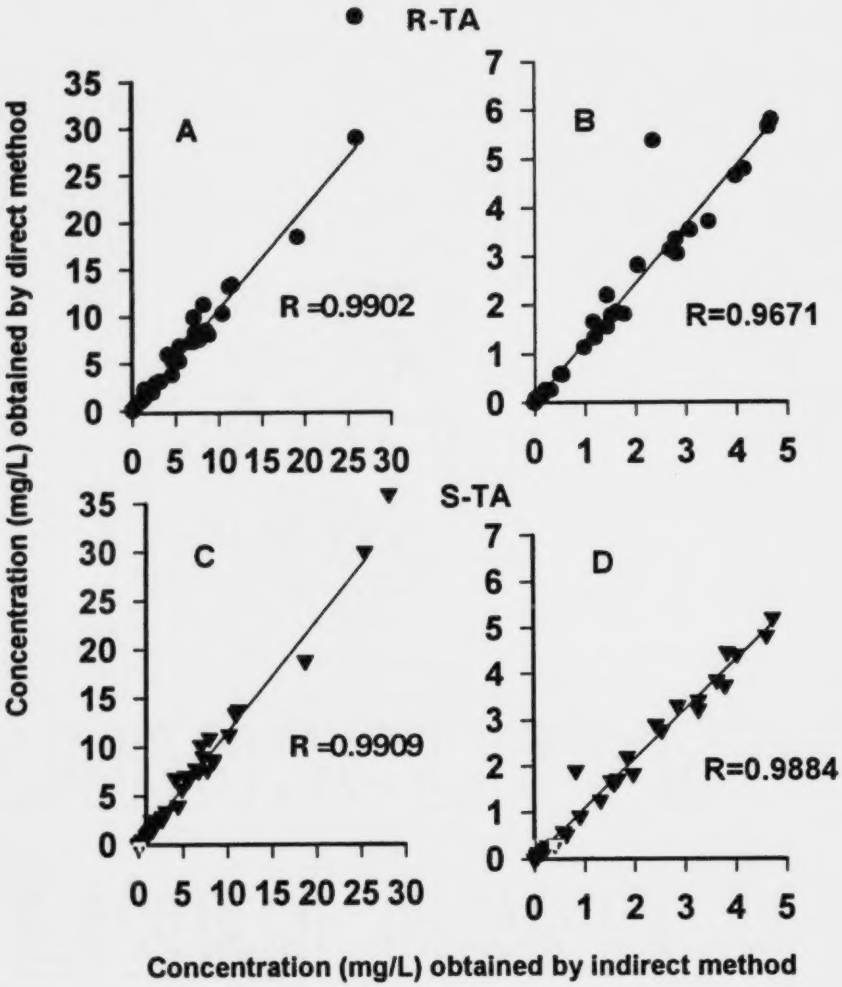

Figure 3.14. **Correlation between concentrations measured by a direct methods *vs.* those measured by an indirect method after administration of regular release tablets (A &C) and sustained release capsules (B & D)**

Table 3.6. Bioavailability Parameters after Oral Administration of Single 300 mg Doses of Racemic Tiaprofenic Acid (Samples Analyzed using Indirect & Direct HPLC Assays)

	Indirect method								Direct method							
	t_{max} (h)		C_{max} (mg/L)		C_{24} (mg/L)		$AUC_{0-\infty}$ (mg.h/L)		t_{max} (h)		C_{max} (mg/L)		C_{24} (mg/L)		$AUC_{0-\infty}$ (mg.h/L)	
Subjects	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
									Regular release formulation							
1	3.0	3.0	11.3	10.9	0.049	0.041	51.1	51.9	3.0	3.0	13.2	13.4	0.066	0.055	56.5	62.6
2	3.0	3.0	10.5	10.2	0.035	0.048	43.7	44.5	3.0	3.0	10.3	11.3	0.037	0.040	41.8	49.6
3	1.5	1.5	19.2	18.8	0.082	0.101	65.4	61.0	1.5	1.5	18.4	18.8	0.060	0.087	64.6	63.8
4	2.0	2.0	26.0	25.6	0.024	0.041	50.6	50.7	2.0	2.0	28.9	30	0.022	0.037	58.1	61.7
Mean	**2.38**	**2.38**	**16.7**	**16.4**	**0.048**	**0.058**	**52.7**	**52.0**	**2.38**	**2.38**	17.7	**18.4**	**0.046**	**0.055**	**55.2**	**59.4**
S.D.	**0.75**	**0.75**	**7.3**	**7.3**	**0.025**	**0.029**	**9.1**	**6.8**	**0.75**	**0.75**	**8.21**	**7.25**	**0.020**	**0.023**	**9.6**	**6.63**
									Sustained release formulation							
1	6.0	6.0	4.74	5.20	0.215	0.254	53.6	53.7	6.0	6.0	4.64	5.64	0.265	0.218	53.0	58.2
2	4.0	4.0	5.14	5.27	0.155	0.173	36.9	38.3	4.0	4.0	4.16	4.11	0.138	0.148	29.3	37.2
3	4.0	4.0	6.07	6.52	0.073	0.107	47.6	54.1	4.0	4.0	5.78	4.19	0.084	0.127	39.2	34.8
4	4.0	4.0	3.84	4.45	0.045	0.073	44.6	47.5	4.0	4.0	4.64	5.79	0.090	0.143	46.9	52.8
Mean	**4.5**	**4.5**[a]	**4.95**[a]	**5.36**[a]	**0.123**	**0.152**	**45.7**	**48.5**	**4.5**[a]	**4.5**[a]	**4.82**[a]	**5.36**[a]	**0.144**	**0.174**[a]	**42.1**	**45.7**
S.D	**1.0**	**1.0**	**0.92**	**0.86**	**0.079**	**0.080**	**6.97**	**7.34**	**1.0**	**1.0**	**0.92**	**0.86**	**0.084**	**0.070**	**10.3**	**7.37**

[a] Significant difference between formulations

Table 3.7. Pharmacokinetic Indices Following Single 300 mg Doses of Regular and Sustained Release Racemic Tiaprofenic Acid (Samples Analyzed using Indirect & Direct HPLC Assays)

	Indirect method						Direct method					
	CL/F (L/h)		V_d/F (L)		$t_{1/2}$ (h)		CL/F (L/h)		V_d/F (L)		$t_{1/2}$ (h)	
Subjects	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
						Regular release						
1	2.94	2.89	8.98	7.90	2.12	1.90	2.66	2.39	8.65	6.57	2.26	1.90
2	3.43	3.37	11.5	12.8	2.31	2.63	3.59	3.03	7.94	7.72	1.53	1.70
3	2.30	2.46	5.06	5.77	1.53	1.62	2.32	2.35	11.7	11.1	3.50	3.24
4	2.96	2.96	7.06	8.07	1.65	1.89	2.58	2.43	4.92	4.17	1.32	1.19
Mean	**2.91**	**2.92**	**8.13**	**8.63**	**1.90**	**2.01**	**2.74**	**2.55**	**8.31**	**7.39**	**2.15**	**2.03**
S.D.	**0.47**	**0.37**	**2.73**	**2.97**	**0.37**	**0.43**	**0.55**	**0.32**	**2.80**	**2.88**	**0.98**	**0.88**
						Sustained release						
1	2.80	2.79	17.6	17.6	4.36	4.40	2.83	2.58	16.6	15.2	4.08	4.08
2	4.07	3.92	19.0	16.2	3.24	2.86	5.13	4.64	12.2	16.8	4.36	2.88
3	3.15	2.77	6.46	4.93	1.42	1.23	3.82	2.74	13.3	14.9	2.41	1.23
4	3.37	3.13	8.50	7.74	1.75	1.71	3.20	2.84	6.98	5.50	1.51	1.34
Mean	**3.35**	**3.15**	**12.90**	**11.6**	**2.69**	**2.54**	**3.74**	**3.05**	**12.3**	**13.1**	**3.09**	**2.38**
S.D.	**0.54**	**0.53**	**6.34**	**6.22**	**1.36**	**1.39**	**1.01**	**0.67**	**4.01**	**5.13**	**1.36**	**1.37**

3.6. *In vivo* studies in rats

3.6.1. Pharmacokinetics of tiaprofenic acid enantiomers in rats

Following iv administration of TA, stereoselective plasma concentration-time profiles were observed (Fig. 3.15A). In general, the concentration of active S enantiomer was significantly higher than its antipode. The difference between $t_{1/2}$ of enantiomers was not significant while the systemic CL and V_d of R-TA were significantly greater than those observed for S-TA (Table 3.8).

Table 3.8. Pharmacokinetic indices following 20 mg/kg iv bolus of S-TA to rats.

	$t_{1/2}$ (h)		CL (mL/h)		V_d (mL)	
	R-TA	S-TA	R-TA	S-TA	R-TA	S-TA
Mean	4.64	4.30	8.84	4.55[a]	55.9	27.2[a]
S.D.	1.24	0.95	2.49	1.50	10.7	7.31

[a] The observed value for S-TA is significantly different than that of R-TA.

Significant stereoselectivity in the plasma concentration *vs* time profiles of the enantiomers was observed following single oral doses of 20 mg/kg of racemic TA as powder (Fig. 3.15C). The concentration of S-TA was consistently higher than that of R-TA. The t_{max} was longer and C_{max} greater for the S enantiomer. No significant stereoselectivity was observed with respect to the mean terminal $t_{1/2}$ values (Table 3.8).

Table 3.9. Bioavailability data obtained after 20 mg/kg of TA to rats.

	tmax (h)		Cmax (mg//L)		AUC (mg•h/L)		Ratio
	R-TA	S-TA	R-TA	S-TA	R-TA	S-TA	S/R
iv bolus							
Mean	---	---	---	---	342.0	667.5[a]	1.98[d]
S.D.	---	---	---	---	58.9	106.8	0.37
Powder							
Mean	0.83	0.5[a]	45.4	57.3[a]	221.6	353.9[a]	1.61[e]
S.D.	0.16	0.26	6.77	6.99	33.4	55.3	0.07
Inclusion complex							
Mean	1.85[c]	2.45	21.4[c]	26.6[a,c]	103.9	148.4[a]	1.37[b,c]
S.D.	0.41	0.52	2.81	4.25	22.7	33.9	0.128
SR beads							
Mean	1.54[c]	2.11	14.40[c]	22.2[a,c]	109.0	178.3[a]	1.68
S.D.	0.82	0.89	4.58	6.32	26.9	25.11	0.20

[a] . **S-TA significantly different from that of R-TA.**

[b] . **Significantly different than iv.**

[c] . **Significantly different than powder.**

[d] . **Significantly different than inclusion complex.**

[e] . **Significantly different than SR beads.**

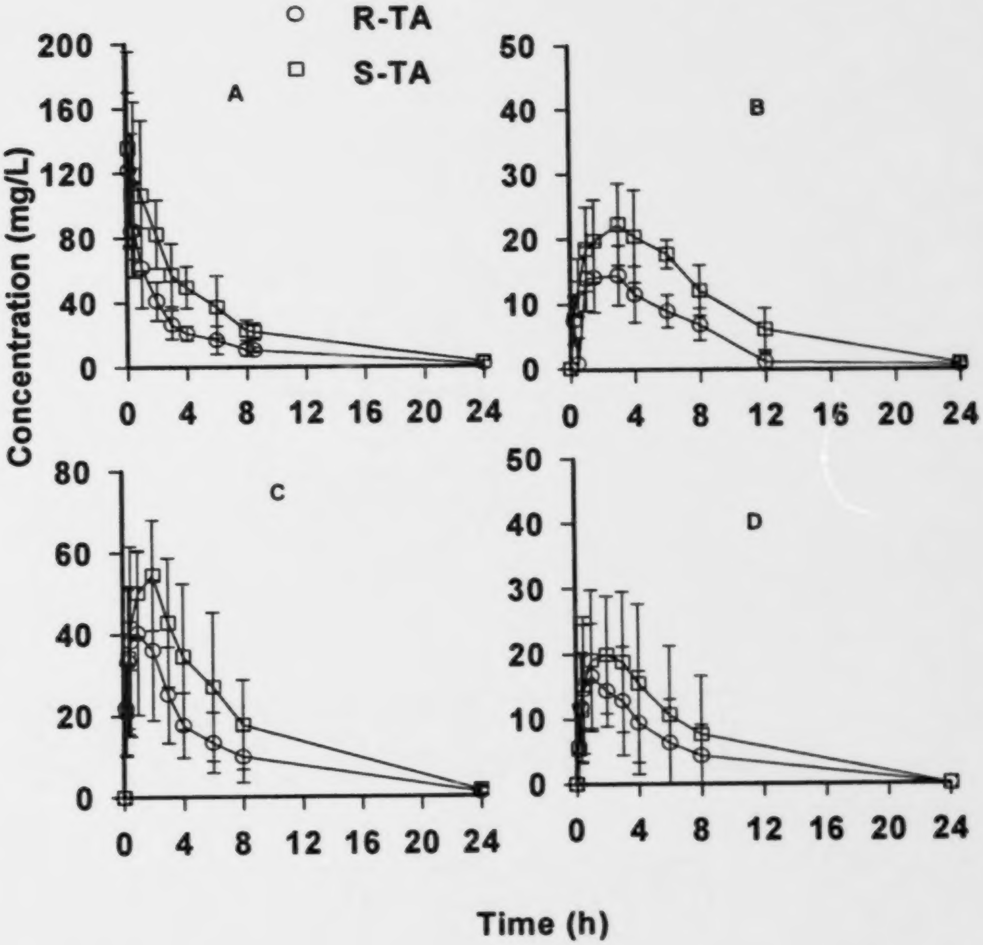

Figure 3.15. Mean plasma concentration-time profiles of R- and S-TA following administration of single 20 mg/kg dose of racemic TA to rats; iv (A) sustained release beads (B), powder (C) and inclusion complex (D)

The absorption rate of enantiomers of TA were significantly reduced after single oral doses of 20 mg/kg of racemic TA given as inclusion complex as reflected by longer t_{max} compared with powder (Table 3.9). Similar to the powder, there was a significant stereoselectivity in the plasma concentrations-time profiles of the enantiomers in favor of S-TA (Fig. 3.15D). There was no difference between S:R ratio-time profiles after powder, inclusion complex, and sustained release beads (Fig. 3.16). The $t_{1/2}$ values for both enantiomers of TA after inclusion complex were not significantly different from those observed after the powder. After inclusion complex, the estimated $AUC_{0-\infty}$ values were significantly lower than those obtained after powder. The mean relative bioavailabilities of TA enantiomers from the inclusion complex were approximately 47% and 42% for R- and S-TA, respectively.

After SR formulation, TA enantiomers were slowly absorbed from the GI tract and the concentration of S-TA was greater than that of R-TA (Fig. 3.15 B). Similar to inclusion complex, extent of absorption was significantly lower after SR beads as compared to that observed after powder. On the other hand, the estimated $AUC_{0-\infty}$ after SR beads was not statistically different than that estimated after administration of inclusion complex.

Following oral administration of the solid dosage forms, absorption of TA was incomplete (Table 3.9). Therefore, in order to establish the same extent of systemic availability, the required doses for toxicity comparisons of various formulations were corrected accordingly.

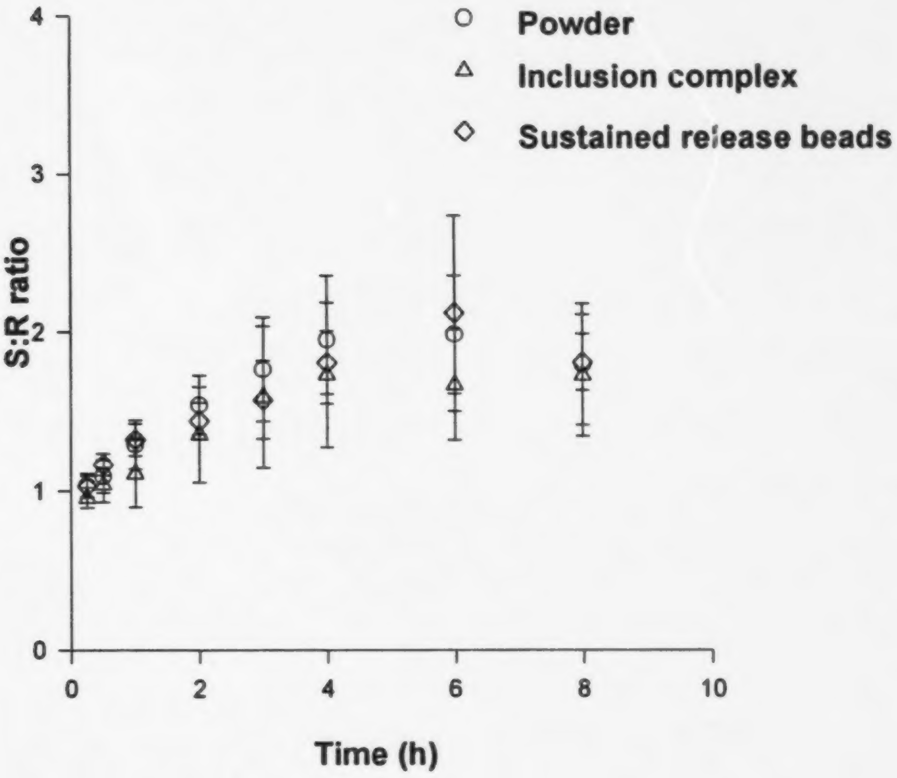

Fig. 3.16. Tiaprofenic acid plasma concentration S:R ratio vs time profiles after powder, TA-DCD inclusion complex, and sustained release beads.

3.6.2. Toxicokinetics of tiaprofenic acid in rats

3.6.2.1. TA-induced intestinal damage

The percent increase in the 0-24 h cumulative urinary excretion ($\Sigma X_{0\text{-}24}$) of ^{51}Cr-EDTA was used as a marker of intestinal permeability. Following oral administration of 20 and 40 mg/kg of racemic TA as a regular release powder and inclusion complex a proportional increase in the intestinal permeability was observed at 3 h post-dose (Fig. 3.17).

Oral administration of DCD did not cause significant damage in the intestine 3 h post-dose. Oral doses of a physical mixture of DCD and TA resulted in damage comparable to that observed after oral administration of the powder (corrected for relative bioavailability of inclusion complex) (Fig. 3.18). Inlcusion complex caused significantly higher damage than powder. Although the intestinal damage after the physical mixture was lower than that observed after inclusion complex, this difference did not reach statistical significance due to a very large variability in the intestinal permeability after administration of the physical mixture (Fig. 3.18).

Fig. 3.19 depicts the time course of the increased intestinal permeability obtained after oral administration of the various formulations of TA. Both modified release dosage forms (i.e., sustained release and inclusion complex) caused more damage than powder. After the administration of sustained release, the area under (%)$\Sigma_{0\text{-}24}$ of ^{51}Cr-EDTA-time curves from 0-24 h (AUC_{PD}) was greater compared to that of the inclusion complex but this differencewas not statistically significant (inclusion complex: 1510±713 and sustained release: 2360±789).

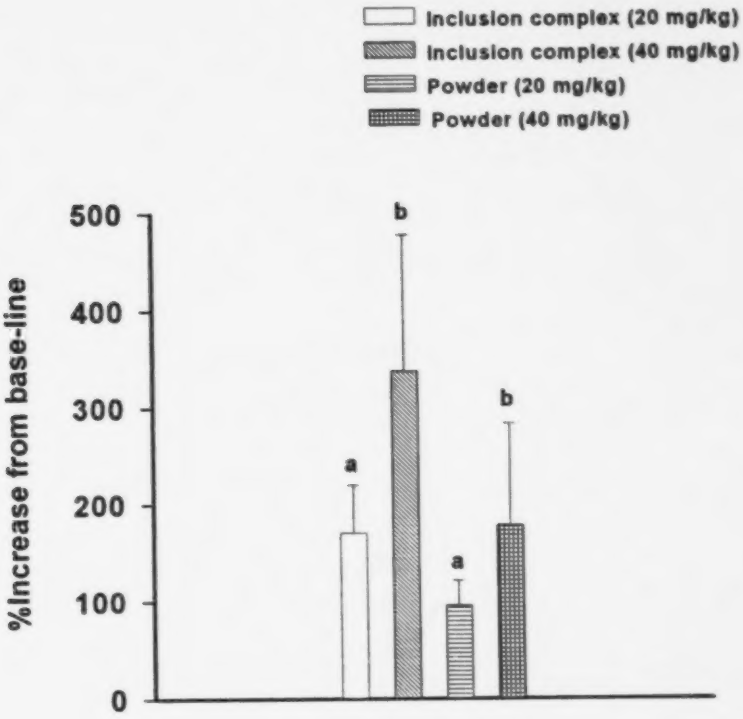

Figure 3.17. %Increase in the intestinal permeability from the base-line after 20 and 40 mg/kg oral dose of TA as powder and inclusion complex at 3 h post-dose.

[a] Significant difference from the observed base-line intestinal permeability value.

[b] Significant difference from the corresponding intestinal value after 20 mg/kg dose of powder

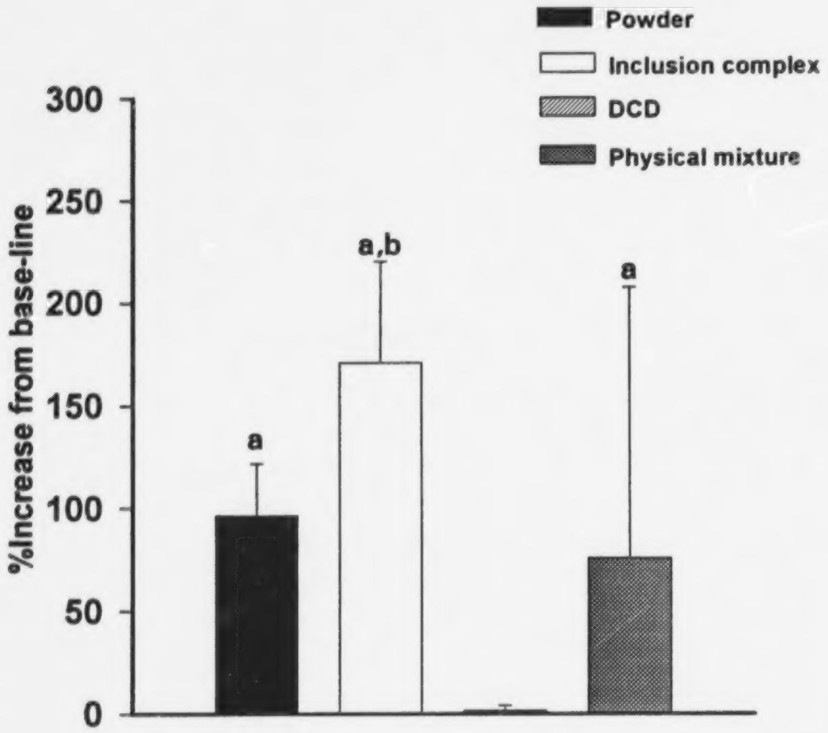

Figure 3.18. **%Increase in the intestinal permeability from the base-line after equivalent to 20 mg/kg oral dose of TA as powder, inclusion complex, DCD, and physical mixture.**

[a]**Significant difference from the observed base-line intestinal permeability value.**

[b]**Significant difference from the corresponding intestinal value after 20 mg/kg dose of powder. (statistical differences assessed using one way ANOVA followed by Duncan's multiple-range test).**

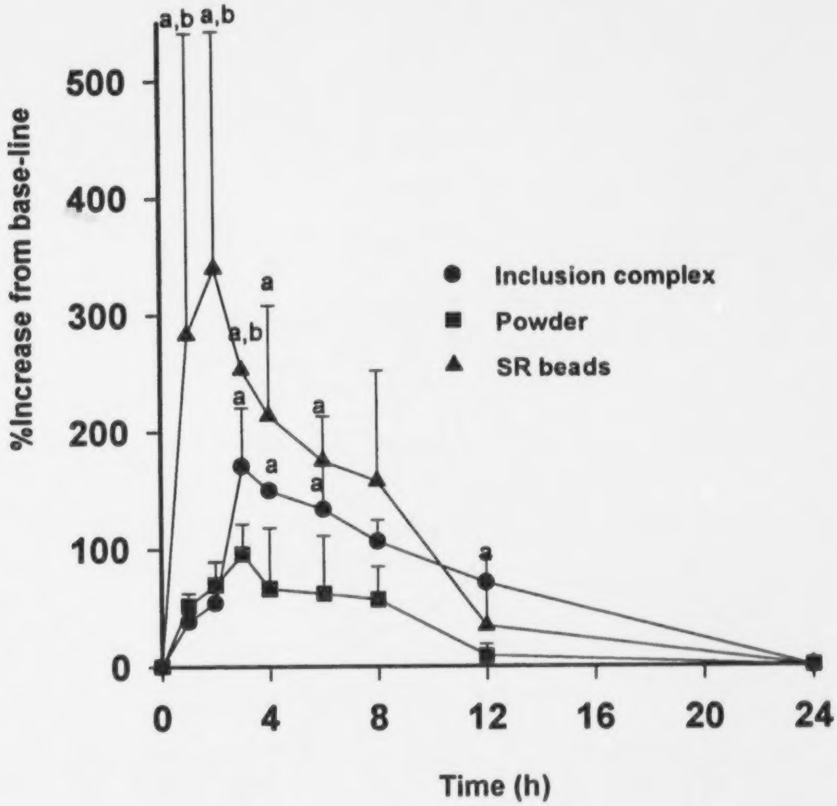

Figure 3.19. The time course of %increase in the intestinal permeability from the base-line after powder, inclusion complex, and sustained release formulation. The letters (a) and (b) represent significant differences between powder and inclusion complex, respectively.

However, AUC_{PD} values after both sustained release and inclusion complex dosage forms were significantly greater than those estimated after iv bolus (228±400) and regular release powder (631±90) (statistical differences assessed using one way ANOVA followed by Duncan's multiple-range test).

Fig. 3.20. depicts a plot of % increase in $\Sigma_{0\text{-}24}$ of ^{51}Cr-EDTA from base-line *vs* plasma concentration of active enantiomer, S-TA. An anti-clockwise hysteresis was observed for all three formulations. The anti-clockwise hysteresis was however less apparent after SR as compared to the powder and inclusion complex. Similarly, an anti-clockwise hysteresis was obtained when % increase in $\Sigma_{0\text{-}24}$ of ^{51}Cr-EDTA from base-line was plotted against the total concentration of TA (R+S) in plasma (Fig. 3.20).

Using the effect compartment model proposed by Holford and Sheiner (1981) and Sheiner (1979) (Fig. 3.21A), the observed anti-clockwise hysteresis was collapsed (K_{eo}:0.7 h^{-1}) (Fig. 3.22). However, the slopes of the lines obtained after collapsing the hysteresis were not the same for all three formulations. After the sustained release and inclusion complex, there was an apparent shift to the left in GI permeability *vr* concentration of drug in the effect compartment (C_e) curves (Fig. 3.22). By employing the DINP (direct input) model (Fig 3.21B), a better relationship between C_e and GI toxicity was obtained after sustained release (K_0: 0.00044 mg/h for S-TA and K_0: 0.00072 mg/h for the total drug) and inclusion complex (K_0: 0.00035 mg/h for S-TA and K_0: 0.0006 mg/h for the total drug) formulations. A plot of percent change in the intestinal permeability *vs* C_e is shown in Fig. 3.23.

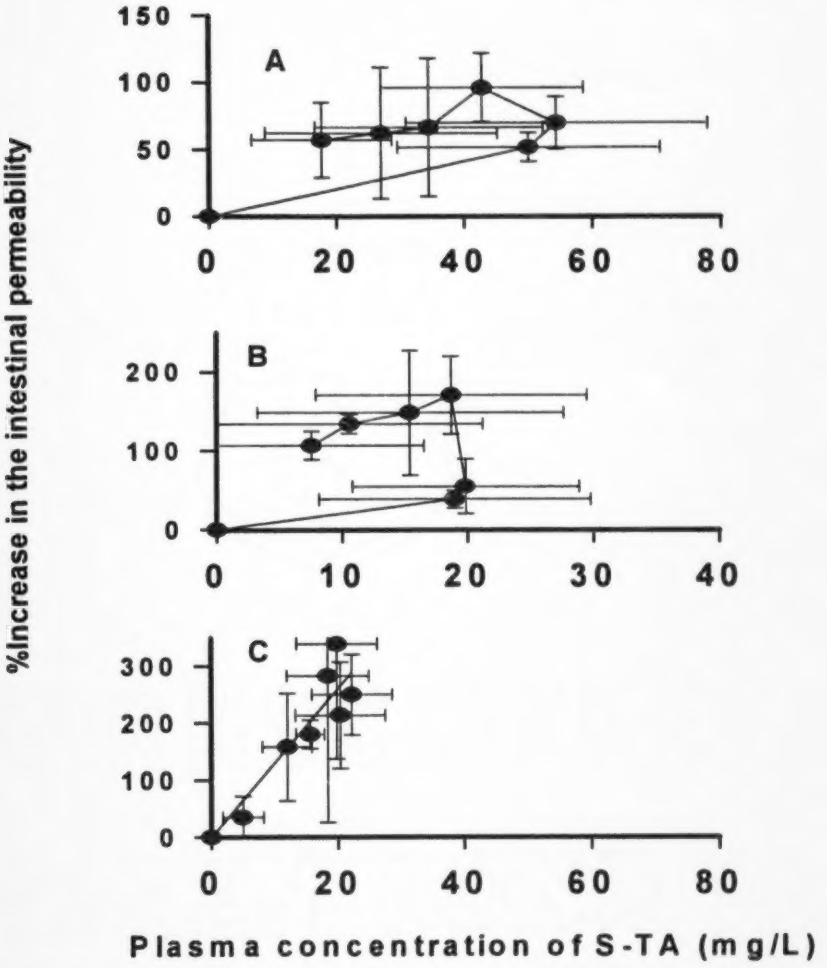

Figure 3.20. **% Increase in the intestinal permeability from the base-line vs plasma concentration of S-TA; powder (A), inclusion complex (B), sustained release (C)**

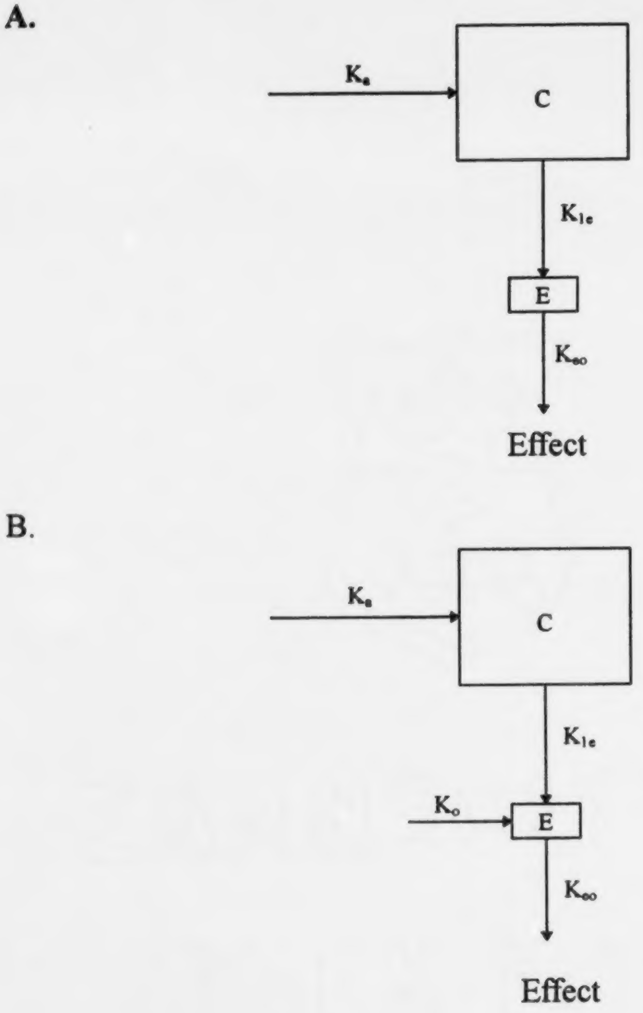

Figure 3.21. Link PK-PD model (A) and PK-PD mode with direct input to the effect compartment (B).

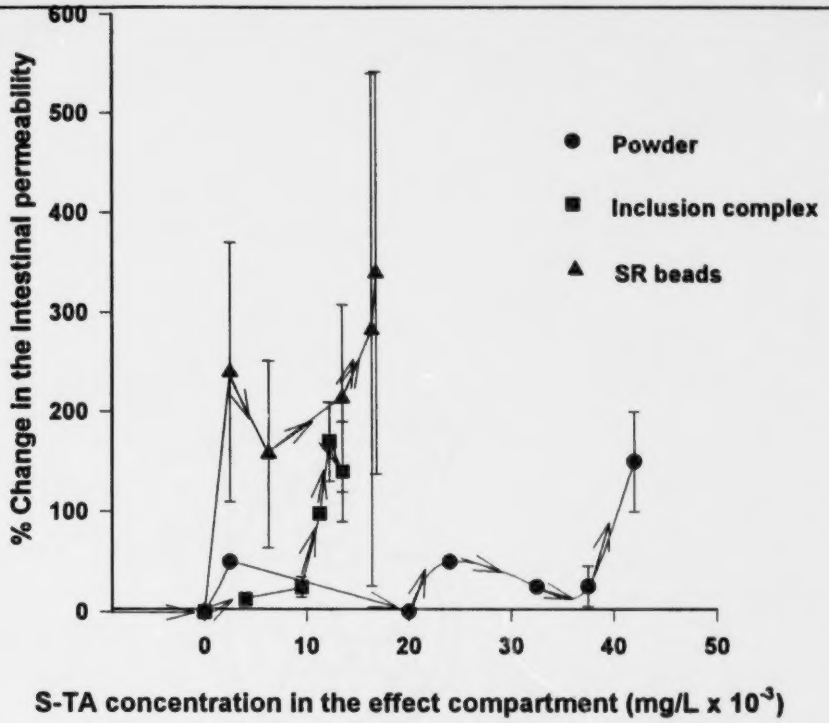

Figure 3.22. % Increase in the intestinal permeability from base-line *vs* the concentration of S-TA in the effect compartment [estimated using the effect compartment model proposed by Holford and Sheiner (1981)].

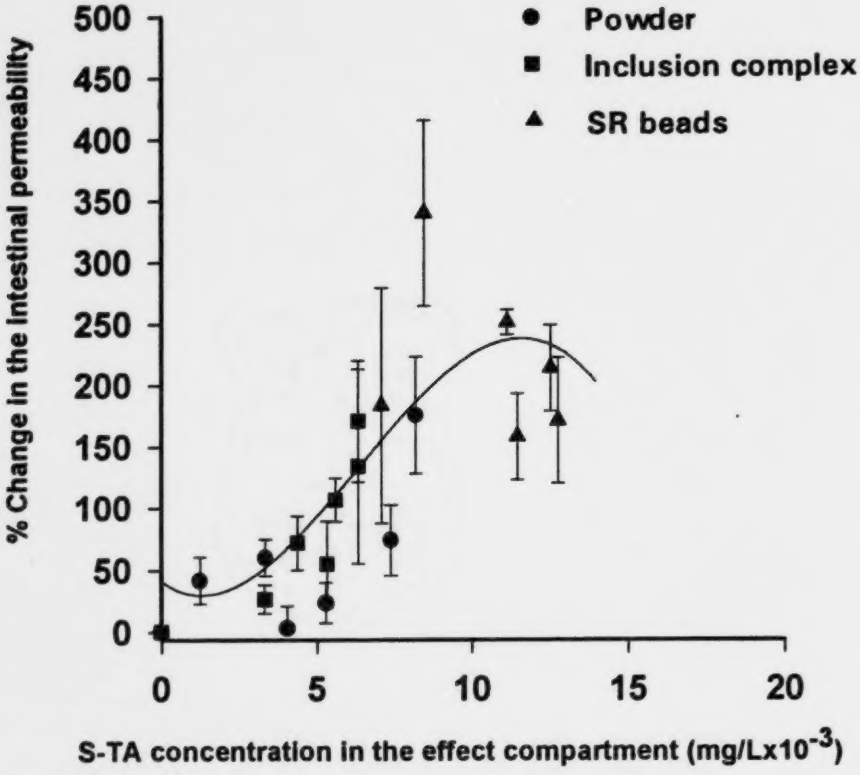

Figure 3.23. % Increase in the intestinal permeability from the base-line vs the concentration of S-TA in the effect compartment (estimated using the modified model with a direct input to the effect compartment)

After fitting C_e vs observed GI toxicity data to a sigmoid E_{max} model, the relevant PD parameters were estimated for both active S-TA and total drug (R+S) (Table 3.10). The quality of fit was evaluated using criteria proposed by Akaike and Schwartz (Akaike, 1978 and Schwartz, 1978). Therefore, it seems that a superior fit results when total concentration of TA in the effect compartment is considered.

Table 3.10. Estimated pharmacodynamic indices for the intestinal damage caused by tiaprofenic acid.

	E_{max}	EC_{50} (μM)	γ
A. Parameters obtained using total concentration (R+S) in the effect compartment:			
Estimated value	186	55	11
S.E.	22	2.4	5.0
95% Confidence interval	138-234	49-60	7.2-28
B. Parameters obtained using concentration of S-TA in the effect compartment:			
Estimated value	214	28	3.0
S.E.	65	7.3	1.61
95% Confidence interval	74-353	12-44	-0.41-6.5

3.6.2.2. TA-induced upper GI damage

The permeability of sucrose after administration of powder was significantly higher than the base-line as depicted in Fig. 3.23. The observed upper GI damage was significantly lower after sustained release (5.1±52.1) and inclusion complex (7.6±12.4) than after regular release powder (197.5±109.6) (Fig. 3.23).

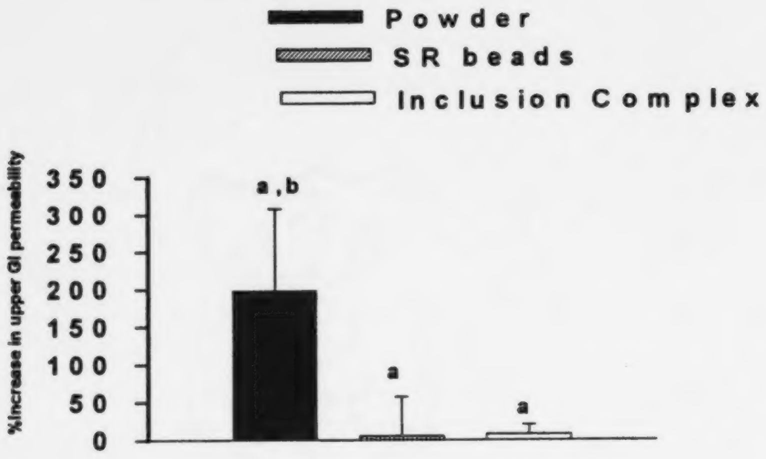

Figure 3.23. % Increase in the upper GI permeability from the base-line (1 h post dose) after powder, sustained release beads and inclusion complex.

4. DISCUSSION

4.1. Direct stereospecific HPLC assay for analyzing TA enantiomers in human plasma

The direct stereospecific HPLC assay developed during the course of this study is a sensitive method for analysis of TA (Fig. 3.1). It provides an acceptable accuracy and precision as reflected by low % error and inter-day % CV values (Table 3.1). This method involves a substantially shorter sample preparation time compared to the previously reported indirect method (Mehvar *et al.*, 1988). The enantiomers of TA were directly resolved previously on a human serum albumin column [HSA-CSP (SFCC-Shandon, Eragny, France). Although protein based columns offer good resolution for the enantiomers of chiral compounds, they lack durability and are expensive (Muller *et al.*, 1993). The CHIRAL PACK AD column employed in the direct HPLC method reported here was cost-effective and exhibited remarkable stability (over 1000 biological samples were injected on one column with no loss of efficiency). Consequently, the reported direct method is suitable for quantification of TA enantiomers in plasma following administration of recommended therapeutic doses.

4.1.1. Enantiomeric conversion and stability of TA enantiomers under chromatographic conditions employed in both direct and indirect methods

Unavailability of the pure enantiomers renders evaluation of Enantiomeric conversion complicated or even impossible. This may explain why the potential for Enantiomeric conversion was not properly addressed during the development of the indirect HPLC methods reported earlier (Mehvar *et al.*, 1988 and Singh *et al.*, 1986).

However, due to recent technological advances, the optical purification of the enantiomers has been simplified. An enantiomeric enrichment technique was employed to obtain 98.6% optically pure TA enantiomer the composition of which remained virtually unchanged over a 24 h period under the conditions used for sample preparation by the direct method. In contrast, when the same enriched enantiomer was subjected to derivatization with L-leucinamide, a small but significant increase in the level of optical impurity was observed which corresponded to 6.955±3.06% chiral conversion. This result is similar to the recent observation of Hutt *et al.* (1994) suggesting a partial Enantiomeric conversion of 15% for TA enantiomers (determined by NMR, and optical rotation) when 2,2,2-trichloroethyl chloroformate and L-Leucinamide were used as coupling and derivatization reagent, respectively. Formation of the diasteriomers by the mixed anhydride method involves reaction of the TA enantiomers with 2,2,2-trichloroethyl chloroformate in the presence of a strong base, 4-dimethylaminopyridine, followed by reaction of the formed mixed anhydride with L-Leucinamide in the presence of triethylamine (Mehvar *et al.*, 1988). A limitation previously reported for this technique was the possibility of partial or complete racemization of the analyte during the derivatization reaction (Wright and Jamali, 1993 and Vakily *et al.*, 1995). It has been suggested that the organic base used in the reaction may be responsible for the observed partial Enantiomeric conversion. Indeed replacement of triethylamine with N-methylmorpholine significantly reduced the observed Enantiomeric conversion (Hutt *et al.*, 1994). Partial Enantiomeric conversion has also been reported for other NSAIDs. Ketorolac enantiomers underwent complete racemization when the mixed anhydride method was used (Vakily *et al.*, 1995). Tiaprofenic acid enantiomers exhibit the second highest degree of partial Enantiomeric conversion during the reaction, followed by flurbiprofen enantiomers (3-6% chiral conversion) (Wright and Jamali, 1993). In addition to organic base, it seems that the concentration of the ethyl chloroformate is also an important factor in the degree of Enantiomeric conversion by the mixed anhydride method. It has been demonstrated that the lower the concentration of ethyl chloroformate the higher the degree of Enantiomeric conversion. In the presence of a significant excess of ethyl chloroformate the derivatization proceeds so quickly that very little unreacted drug is available for Enantiomeric conversion. On the other hand, some drugs such as ketoprofen and etodolac exhibit a high degree of optical stability during the pre-column derivatization process (Wright and Jamali, 1993).

Both direct and indirect methods may involve sample preparation and derivatization processes which may render the molecule stereochemically unstable. For example, ketorolac undergoes complete and instantaneous racemization in a medium with an high alkalinity and ionic strength (Vakily *et al.*, 1995).

In developing chiral assays, therefore, consideration should be given to the possibility of stereochemical instability of the enantiomers. It is extremely important to investigate the possibility of partial or complete racemization of the enantiomers when developing a new stereospecific assay regardless of the method of resolution. Altered enantiomeric composition of the samples due to Enantiomeric conversion renders interpretation of pharmacokinetic studies complicated and may result in erroneous conclusions.

It has been suggested that TA may undergo photo-degradation which may in turn create further complications during sample analysis. Therefore, the stability of TA enantiomers (5mg/L) in a pH 7.4 buffer system was evaluated under normal laboratory illumination (fluorescent lighting). The concentrations of TA enantiomers slowly decreased following first order kinetics with a $t_{50\%}$ of 13 days. It is worth of mention that samples were collected for only 5 days and, considering the slow rate of degradation, estimation of the $t_{50\%}$ may not be accurate. Similarly, Jamali *et al.* (1984) have observed a similar stability profile for TA enantiomers in plasma, urine, and methanol under normal laboratory conditions. Nevertheless, it seems the molecule does not undergo significant degradation during sample preparation described for both direct and indirect methods. Simple light precautions such as aluminum foil covering of the samples and/or use of amber colored tubes and inserts are sufficient to prevent photo-degradation.

4.1.2. Reconfirmation of the previously reported pharmacokinetic data of tiaprofenic acid

Independent of the analytical method (direct or indirect) used for analyzing the plasma samples, similar plasma concentration-time profiles were obtained for both enantiomers of TA after oral administration of conventional and SR formulation (Fig. 3.13). Both R- and S-TA were eliminated rapidly with $t_{1/2}$ of 2 h (Table 3.7). Typical of this group of acidic agents with very high plasma protein binding, a small apparent volume distribution was observed for TA enantiomers. There was no significant difference between pharmacokinetic indices calculated for the individual enantiomers.

The results obtained here are in close agreement with those previously observed during assessment of the *in vivo* performance of the sustained release and regular release formulations indicating that the plasma concentration-time courses of TA enantiomers are not significantly different despite the observed small chiral conversion using the indirect method (Fig. 3.12 and Tables 3.4 and 3.5). Since there was an excellent correlation between measured concentration using direct and indirect method for both regular tablet formulations, it seems that both analytical methods are suitable for analyzing samples from pharmacokinetic studies. On the other hand, Muller *et al.* (1993) have reported stereoselectivity in the pharmacokinetics of TA. The discrepancy between the results obtained here and those reported by Muller *et al.* may be attributed to the small number of plasma samples (n=3), and the lack of proper statistical analysis to prove unequivocal the presence or absence of stereoselectivity in the pharmacokinetics of TA enantiomers (Muller *et al.*, 1993). Furthermore, a comparison of data presented by Muller *et al.* (1993) revealed no significant stereoselectivity in plasma concentrations of the enantiomers. They have also reported the pharmacokinetics for TA to be stereoselective in rats using their direct HPLC method (Muller *et al.* 1993). Similar results have been observed using the indirect method indicating its capability of detecting the differences between the enantiomers of TA (Fig. 3.15 and Tables 3.8 and 3.9).

Therefore, the *in vitro* release and *in vivo* pharmacokinetic data obtained during the evaluation of the possibilty of stereoselective release of TA enantiomers from various formulations are valid when the pre-column dervitization technique (Mehvar *et al.*, 1988) was employed to resolve the TA enantiomers.

4.2. Stereoselective release and *in vivo* performance of 2-APA enantiomers from formulations containing chiral excipients

The release of 2-APA (TA and FB) enantiomers from the selected formulations exhibited formulation, pH and drug dependent stereoselectivity. The mechanism of such stereoselective release may be attributed to the intermolecular interactions between the individual enantiomers and the chiral excipient(s) included in the formulation of the sustained release dosage forms (Daniel *et al.*, 1986 and Imai *et al.*, 1988). During a rapid and complete release/dissolution process, the enantiomers were completely dissolved in the dissolution medium in a short time. It was, therefore, difficult to detect any stereoselectivity in the release. The slow release and dissolution of enantiomers in the aqueous medium from the sustained release formulations used in this study, however, might provide sufficient time for the stereospecific interaction of enantiomers with the matrix utilized in the formulation of the sustained release products (Horiuchi *et al.*, 1990, Hirayama, *et al.*, 1993, Duddu *et al.*, 1993 and Aubry *et al.*, 1993). During dissolution water penetrates and dissolves the racemic compound included in the matrix. Subsequently dissolved enantiomers may interact with matrix by forming weak bonds (e.g. hydrogen bonds) which are important in the chiral recognition and stereoselective release (Lipkowitz *et al.*, 1993 and Duddu *et al.*,1993).

4.2.1. *In vitro* release of flurbiprofen from CM-chitin coated DPPC liposomes, HPMC-alginate, and HPMC-alginate-DMCD formulations

Chiral polysaccharides such as CM-chitin are used to improve the stability of liposomes and reduce the rate of release from the formulation (Dong and Rogers, 1991). Chiral coating materials may preferentially affect the release of enantiomers of racemic compounds included in the liposomal formulation. However, the release of FB from the DPPC liposomes coated with CM-chitin did not exhibit stereoselectivity at pH 7.4 (Fig. 3.5 and 3.6). Altering the degree of ionization of flurbiprofen during the preparation of the liposomes did not result in stereoselective interaction or release from the formulation (Fig. 3.6). Regardless of whether flurbiprofen molecules are trapped in the inner aqueous compartment of liposomes or included within the hydrophobic region of the phospholipid bilayers, there was no apparent difference in the release profiles of flurbiprofen enantiomers. Although reducing the pH of aqueous phase used in the preparation of liposomes significantly increased the extent of encapsulation (Fig. 3.7), the greater loading of the liposomes did not affect stereoselectivity in the release profiles of flurbiprofen enantiomers.

Stereoselectivity in the release of propranolol enantiomers from an HPMC matrix has previously been reported (Duddu *et al.*, 1993). However, significant differences in the release profiles of flurbrprofen enantiomers from the HPMC-alginate sustained release beads could not be found. In addition, incorporation of DMCD in the formulation did not result in stereoselective release of the drug from the formulation. DMCD acts as a solublizing agent and significantly increased the release rate of flurbiprofen from the formulation rendering detection of stereoselectivity (if any) difficult.

4.2.2. *In vitro* and *in vivo* evaluation of a commercially-available sustained release formulation of tiaprofenic acid

The release of TA from the sustained release capsules [excipients: glycerol monostearate, talcum BP, and microcrystalline cellulose BP (Roussel, Montreal, Canada)] exhibited small but significant stereoselectivity. Dissolution tests performed on the sustained release capsules at pH 7.4, demonstrated stereoselective release with 1.5-29% greater cumulative release after 24 h for R-TA than for S-TA (Fig. 3.9B). This observation of pH 7.4 may not reflect the *in vivo* conditions because only 66.9% of R-TA and 60.7% of S-TA were dissolved after 24 h, almost the entire dose of the sustained release formulation was absorbed by the subjects (Table 3.4). This indicates a more rapid dissolution *in vivo* than *in vitro*. Tiaprofenic acid is a weak acid with greater solubility at higher pH values. Indeed, when the pH was adjusted to 8 the release increased and no stereoselectivity in the release profile was detected (Fig. 3.9 C). Despite stereoselective release from the sustained release capsules at pH 7.4, the plasma concentration-time profiles of TA enantiomers were superimposable in humans after both regular and sustained release formulations (Fig. 3.12). The lack of stereoselectivity in the pharmacokinetics of TA is in agreement with previous reports (Singh *et al.*, 1986) (Tables 3.4 and 3.5). The plasma concentration-time data following the regular formulation was described by using a two compartment open model. In contrast, the plasma concentration-time data after sustained release formulation had the characteristics of a one-compartment open model. The above discrepancy may be explained by prolonged absorption from the sustained release capsules which masks the distribution phase resulting in an apparent monoexponential decline (Gibaldi, 1982). TA, similar to some other 2-APA derivatives (e.g. ketoprofen), undergoes extensive glucuronidation (Jamali *et al.*, 1989 and Singh *et al.*, 1986). The small amount of intact drug observed in urine is very likely a product of spontaneous hydrolysis of the glucoronide either during residence in the bladder or during the storage period.

In both rats (Simmonds *et al.*, 1980, Foster and Jamali, 1988, and Berry and Jamali, 1991) and humans (Jamali *et al.*, 1988, Borin *et al.*, 1988, and Jamali *et al.*, 1992), the GI tract has been suggested as a site of unidirectional inversion of 2-APA (R to S enantiomer) particularly following administration of formulations giving slow rate of absorption. Therefore, despite the observed lack of inversion after regular release formulations, a sustained release product of TA may exhibit stereoselectivity due to chiral inversion in the GI tract. However, despite the significant prolongation of t_{max} after sustained release capsules of TA (1.5 h after tablets *vs* 4.0 after sustained release), no significant stereoselectivity was observed in the plasma concentration-time profiles of TA. This indicates that, in humans TA does not undergo any significant chiral inversion even if it resides in the GI tract for a relatively long period of time. Based on the plasma drug concentration data, however, the presence of stereoselective release in favor of R-TA and simultaneous presystemic chiral inversion due to the long residence time in the GI tract can not be ruled out. Stereoselectivity in the release of R-TA is expected to result in a higher plasma concentration of the enantiomer at least in the earlier portion of the absorptive phase. This stereoselectivity during release, however, may be masked by a simultaneous chiral inversion in the GI tract. After administration of sustained-release formulation, it was noticed that *in vitro* release in favor of R-TA (Fig. 3.9B), the enantiomer which is shown to be a substrate specific for chiral inversion of the 2-APA NSAIDs. The lack of a significant difference in 24 h cumulative urinary excretions of TA enantiomers after both regular and sustained release formulations (Table 3.4) supports the suggestion that TA does not undergo chiral inversion in humans, regardless of the rate of absorption.

4.2.3. *In vitro* and *in vivo* evaluation of DCD and tiaprofenic acid inclusion complex

The preparation of partially substituted ß-cyclodextrin such as heptakis-(2,6-di-O-methyl)-ß-cyclodextrin and heptakis-(2,3,6-tri-O-methyl)-ß-cyclodextrin has been previously reported (Szejtli *et al.*, 1980). These methylated ß-cyclodextrin derivatives are extremely water soluble and highly surface active (Uekama, 1985). As mentioned above, rapid dissolution of 2-APA after complexation with ß-cyclodextrin and its hydrophilic derivatives may mask stereoselectivity in release as the entire amounts of both enantiomers are very rapidly released. However, when a more hydrophobic substituted ß-cyclodextrin such as heptakis-(2,6-di-O-ethyl)-ß-cyclodextrin (DCD) is used for the preparation of the inclusion complex, a significant reduction in the release rate of enantiomers of a racemic compound may become apparent. This reduction may provide a means for a proper assessment of any differences in the release characteristics of enantiomers. Therefore, DCD was synthesized with a modification of the method employed for methylating anhydrous ß-cyclodextrin (Szejtli *et al.*, 1980). The ethylation of ß-cyclodextrin was accomplished in the presence of BaO and $Ba(OH)_2$. $8H_2O$ with diethyl sulfate as an alkylating reagent. The yield of the reaction was 74% on the bases of all of the product was DCD. Although it is possible that the final product is a mixture of mono, di and/or tri-ethyl-ß-cyclodextrins, the proton and carbon NMR spectra indicate that heptakis-(2,6-di-O-ethyl)-ß-cyclodextrin is the main component. Furthermore, comparison of our NMR spectra with those of authentic heptakis-(2,6-di-O-ethyl)-ß-cyclodextrin (Hirayama *et al.*, 1993) confirmed the above conclusion. Consequently, further attempts were not made to isolate DCD from the other substituted ß-cyclodextrins, since the objective of this work was reduction of the release rate of the enantiomers and evaluation of the difference in the release rate of R- and S-TA from the DCD inclusion complex.

It has been demonstrated that S-flurbiprofen is preferentially included in the cavity of trimethyl-β-cyclodextrin (Uekama, 1985 and Imai *et al.*, 1988). This interaction has been attributed to changes in the capacity for intramolecular hydrogen bonding due to the replacement of the hydroxyl by methoxy groups (Uekama, 1985 and Imai *et al.*, 1988). In a recently published study, the difference in the binding enthalpy, (ΔH), was determined for the individual enantiomers of fenoprofen, flurbiprofen, 1-phenylethanol, and mandelic acid. An enhanced enantioselectivity in favor of the S enantiomer of the aforementioned drugs (high difference in binding enthalpy values) was observed when the alkylated cyclodextrins were employed as host molecules. This difference in enantiodiscriminating ability (i.e. derivatized vs. native cyclodextrins) was attributed to significant intermolecular contact with the exterior of the derivatized cyclodextrins whereas there is only interior contact with the native cyclodextrins. Therefore, it seems that chiral discrimination may take place on the exterior of cyclodextrins (Lipkowitz *et al.*, 1993). We have similarly observed the preferential inclusion of S-TA into DCD. A slower rate of release and longer lag-time for S-TA was evident suggesting preferential interactions and/or release of this enantiomer with DCD. Stereoselectivity seems to be pH dependent with the largest differences between the enantiomers observed at pH 3.0 (S:R ΣR_{24} ratio: 0.88±0.04) (Fig. 3.11A). Moreover, elevation of the pH to 7.4 significantly reduced stereoselectivity. This may be explained by the faster dissolution of TA as a result of increased ionization at higher pH. Indeed, the influence of pH on the release rate of various NSAIDs from cyclodextrin has been reported (Zecchi *et al.*, 1988 and Orienti *et al.*, 1989). It appears that elevation in pH of the dissolution medium results in a decrease in the apparent stability constants of NSAIDs-cyclodextrin inclusion complexes (Orienti *et al.*, 1989). We similarly observed a pH-dependent rate of release of TA enantiomers from both the powder and DCD inclusion complex. Complexation resulted in a significant reduction in the rate of dissolution and 24 h cumulative recovery of the enantiomers at pH values of 1.5 and 3.0. The release at pH 7.4, on the other hand, was rapid from the inclusion complex. In contrast, the slow release and dissolution of diltiazem HCl, a water soluble drug, from DCD inclusion complex did not appear to be affected by pH, agitation, or low surfactant concentrations (Yasuhide *et al.*, 1990).

Both the extent and the rate of release from DCD appear to increase with elevation of pH with rapid and complete release at pH 7.4 (Fig. 3.11B). This suggests that the prolonged t_{max} observed after oral administration of the inclusion complex (Fig. 3.15D) is due to slow release in the stomach and duodenum where low pH ranges are expected.

Once in the distal intestine, the drug appears to be released from the formulation and absorbed immediately and completely. Comparison of TA plasma concentration S:R ratios *vs* time curves after inclusion complex, sustained release beads, and powder indicate no *in vivo* differences in the stereoselectivity of release between these 3 formulations (Fig. 3.16). It is not surprising, therefore, that no significant *in vivo* stereoselectivity was observed since the major site of release after inclusion complex administration was the distal intestine where the pH is alkaline and no stereoselectivity in release was detected at pH 7.4. Although S:R AUC ratio after inclusion complex was significantly lower than that observed after administration of TA as iv and powder, any stereoselectivity in the release and subsequent absorption of the enantiomer was not clearly evident due to complicating factors such as *in vivo* variability and the possible presence of presystemic chiral inversion of R-TA (Jamali *et al.*, 1988). Nevertheless, the apparent *in vitro* stereoselectivity at pH 3.0 was not substantial enough to result in appreciable changes in the pharmacokinetics of TA.

After single iv and oral doses of TA in rats, plasma concentrations of the S enantiomer were predominant. This is contrary to the observation in humans where superimposable plasma concentrations were noticed for the enantiomers (Jamali *et al.*, 1988). The mechanism of the observed stereoselectivity of TA pharmacokinetics in rats was not investigated, nevertheless, it may bespeculated to be due to stereoselectivity in the distribution and/or elimination processes. Furthermore, similar to some other 2-APAs, TA in rats may undergo unidirectional chiral R to S inversion (Jamali *et al.*, 1989).

4.3. Formulation dependent TA-induced GI toxicity

The primary reason for the introduction of new formulations of NSAIDs to the market is not only to improve their clinical potency, but to ameliorate the common upper GI tract side-effects associated with these drugs. Enteric coated formulation of acetylsalicylic acid have shown reduced gastroduodenal toxicity by decreasing presystemic toxicity (Aabakken, 1992). Moreover, in recent years considerable attention has been given to pharmaceutical applications of cyclodextrins (Szejtli, 1994, Rainsford, 1990, and Uekama, 1987). Several studies suggest that NSAIDs complexed with β-cyclodextrin can potentially be beneficial in the reduction of upper GI damage (Szejtli, 1994). Gastroduodenal damage caused by piroxicam and naproxen is reduced when these agents are given as a complex with β-cyclodextrin (Rainsford, 1990, Szejtli, 1994, and Lee *et al.*, 1994). This beneficial effect of cyclodextrins has been attributed to their ability to accommodate NSAIDs in their hydrophobic cavity. Consequently, the dissolution rate of NSAIDs and their aqueous solubilities in acidic media (pH 2) was improved (Zecchi *et al.*, 1988). The enhanced dissolution rate results in a substantial improvement in the rate and extent of absorption which in turn reduces the direct contact time of NSAIDs with the upper GI mucosa (Rainsford, 1990, Espinar *et al.*, 1991a, Lee *et al.*, 1994, Patio *et al.*, 1989, and Espinar *et al.*, 1991b). In addition, the modified release formulations of NSAIDs (sustained release and enteric coated) are thought to be effective in decreasing the gastroduodenal damage due to their ability to by-pass the stomach and duodenum and deliver their active ingredient to the more distal portion of the GI tract. This can only be true if one assumes that the GI toxicity of NSAIDs is a site-specific phenomenon and is limited to the upper GI tract. However, there are a growing number of reports indicating rare but serious small intestinal abnormalities associated with NSAID use. A long term use of these drugs may result in considerable blood and protein loss, which may explain the anemia often observed in patients suffering from rheumatoid arthritis (Bjarnason *et al.*, 1987, Bjarnason *et al.*, 1988, Bjarnason *et al.*, 1989, Morris *et al.*, 1992). Furthermore, there are reported cases of NSAID-induced mucosal ulceration and strictures which may require surgical intervention (Bjarnason *et al.*, 1988 and Fellows *et al.*, 1992). Although enteric coated and sustained release formulations may potentially be useful in the reduction of the observed upper GI damage, a shift in the site of toxicity from the proximal to the more distal GI tract may occur. Indeed, a sustained release formulation of indomethacin, Osmosin^R, has been withdrawn due to severe lower intestinal complications with actual perforation. Potassium included in the formulation of this product may have some contribution in the observed intestinal damage. However, the lack of fibrotic changes which is commonly observed in the potassium-induced ulcers suggests that the observed damage was due to delivery of indomethacin to the more distal intestine (Aabakken and Osens, 1989).

The release of TA enantiomers from both inclusion complex and sustained release formulations exhibited a pH dependent rate and stereoselectivity. Increasing pH of dissolution medium resulted in a more rapid and complete release and was associated with no stereoselectivity in release after either formulation. In acidic pH, on the other hand, the release appeared to be stereoselective from the sustained release formulation. Since prostaglandin inhibitory activity is largely attributed to the S enantiomer of 2-APA and absorption of the drug may commence in the upper GI tract (i.e. acidic media), stereoselective release from the formulation may affect the pharmacodynamic characteristics of the drug. Neverthless, our observation indicates that stereoselective release from the inclusion complex and sustained release formulations had no apparent consequence on the pharmacokinetics of TA enantiomers. Since the release of TA enantiomers was more complete with no stereoselectivity at pH 7.4, no change in the pattern of *in vivo* stereoselectivity is expected. The *in vivo* variability in the pharmacokinetics of TA enantiomer may also mask observed *in vitro* stereoselectivity in the release at pH 3. However, despite the lack of stereoselectivity in pharmacokinetics, pH dependent rate and extent of *in vivo* release from the formulation may be of therapeutic consequence. Considering a more complete release of TA enantiomers from the sustained release formulation at higher pH values, it is likely that the more distal segment of the intestine is being exposed to TA after administration of this product. The observed differences in the release characteristics of these 3 dosage forms of TA (regular release powder, inclusion complex, and sustained release) enabled assessment of the influence of formulations on GI toxicity.

Since DCD alone did not cause significant damage of the intestine, the observed increase in the inestinal permeability can be attributed to TA. In addition, doubling the dose of TA from 20 to 40 mg/kg resulted in a proportional increase in the intestinal permeability (Fig. 3.17). This indicates that the observed effect after 20 mg/kg of TA was in the ascending portion of the dose-response curve. Therefore, the selected dose of 20 mg/kg was suitable to discriminate the differences (if any) between the 3 formulations.

Sucrose has previously been utilized as a marker for a noninvasive and site specific assessment of upper GI toxicity in animal models (Medding *et al.*, 1993 and Davies *et al.*, 1995). Sucrose has a limited absorption from the intact upper GI mucosa typical of dissacharides. Following administration of NSAIDs, the integrity of the GI mucosa is altered resulting in an increase in the permeability of sucrose (Medding *et al.*, 1993). Sucrose undergoes a rapid hydrolysis to mono-sugars in the intestine, therefore, the detection of intact sucrose in urine indicates NSAID-induced damage to stomach and duodenum (Medding *et al.*, 1993). The suitability of sucrose as a marker for NSAID-induced gastroduodenal damage has been demonstrated with a good correlation between endoscopic findings and the increased urinary excretion of sucrose (Sutherland *et al.*, 1994). On other hand, ^{51}Cr-EDTA has been utilized as a selective permeability marker for the distal intestine (Bjarnason *et al.*,1984). Since urinary excretion of ^{51}Cr-EDTA correlates with intestinal ulceration, this marker can be used to evaluate NSAID-induced damage to the lower intestine (Davies *et al.* 1994).

After administration of TA powder, the upper GI tract permeability of sucrose was significantly higher than that observed after the inclusion complex and sustained release formulation (Fig. 3.24). This suggests substantial damage to the upper GI tract after regular release powder. This was expected following administration of immediate release formulations due to the exposure of the upper GI tract to a very high amount of NSAID. The rapid absorption of the released drug at the more proximal site of the GI tract spares the distal intestine from direct exposure to the noxious agent. On the other hand, the effect of an immediate release formulation on the lower intestine although significant was very limited likely due to its extenive absorption before reaching the lower gut (Fig.3.19).

In comparison administration of the modified release formulations resulted in very limited gastroduodenal damage (Fig. 3.24). Both the inclusion complex and the sustained release beads caused significant damage to the distal intestine (Fig. 3.19). This suggests that TA induced GI toxicity is formulation-dependent. After iv administration of drug, however, a limited significant increase in the intestinal permeability was also observed. Hence, it is reasonable to assume that there are both a systemic and presystemic components for the TA-induced lower intestinal damage.

As indicated from the observed anti-clockwise hysteresis in the relationship between the plasma concentrations and the intestinal toxicity (Fig. 3.20), there was a lag time between the concentration of drug in the plasma (central compartment) and increase in the intestinal permeability obtained from urinary excretion data of ^{51}Cr-EDTA. Even though the conventional effect compartment model proposed by Holford and Sheiner (1981) (Fig.3.21A) was useful in collapsing the observed anti-clockwise hysteresis, different slopes were found for the collapsed lines of the effect vs TA concentration in the effect compartment after different formulations. There was an apparent shift to the left in the intestinal damage *vs* the drug concentration in the effect compartment curves after the inclusion complex and sustained release products (Fig. 3.22). This indicates that this model does not adequately describe the underlying relationship between concentration and TA-induced intestinal damage for either dosage forms. These products are designed to release their contents in the more distal part of the intestine consequently releasing the drug directly to the site of toxicity (effect compartment). Hence the Holford and Sheiner (1981) model, which assumes a small and deep effect-compartment accessible only through the central compartment, does not hold valid for these types of dosage forms. Alternatively, the effect compartment model of Holford and Sheiner (1981) was modified in order to account for the direct input of TA to the intestinal epithelial cells (effect compartment) following oral administration of the modified release formulations. Using the modified model (Fig. 3.21B), it was possible to obtain a better relationship between the concentration of the drug in the effect compartment and the observed increase in intestinal permeability (Fig. 3.23), further supporting the observation that the TA-induced intestinal damage is highly formulation-dependent.

The presence of so-called inactive or less active enantiomer may substantially affect the net observed toxicological response by either ameliorating or aggravating the clinical outcome (Jamali, 1989). Interestingly, better estimates of pharmacodynamic parameters such as E_{max} and EC_{50} have been obtained when total concentration (R+S) of TA was used instead of only the concentration of the active S-TA. This suggests that the R enantiomer may also have contributed to the observed TA-induced intestinal damage as well. The R enantiomer may cause intestinal damage through prostaglandin independent mechanisms and/or due to its direct irritant effect on the intestinal mucosa (Wright and Jamali, 1993). Unequivocal proof of this, however, requires further study using the individual enantiomers of TA.

4.4. CONCLUSION

Tiaprofenic acid exhibited a pH-dependent rate and stereoselectivity in the release from DCD and commercially available sustained release formulations. Although in this case stereoselectivity in release had no apparent consequence on the pharmacokinetic indices, the possibility of stereoselective release should be considered in the developmental stage of formulation of a racemic drug when optically-active excipients are included in the dosage form. The lack of correlation between *in vitro* dissolution patterns and *in vivo* disposition kinetics has been a subject for debate for many years. Nevertheless, it is imperative to consider and study the possibility of preferential interaction of enantiomers with chiral materials incorporated within the dosage form during the process of formulation as our observation cannot be extrapolated to other classes of drugs. Altered pharmacokinetics of enantiomers due to differential input may also lead to changes in the pharmacodynamic and toxicological profiles of the drug.

TA-induced GI damage exhibited a formulation-dependent pattern. Upper GI toxicity was significant after regular release formulation as compared to that observed after modified release formulations. On the other hand, after oral administration of the sustained release and TA-DCD inclusion complex, the small intestine was the main site of TA-induced damage. Therefore, the possibility of formulation-dependent GI toxicity should be considered in developing new NSAID formulations, with particular attention to the absorption kinetics and its relation to the site of toxicity. Gastric damage caused by

NSAIDs is clinically important and, therefore, a parallel evaluation of lower intestinal toxicity is essential in order to obtain a complete assessment of GI damage.

It seems that the total concentration of TA, as opposed to only the concentration of S-TA, is a better predictor of GI tract damage. This may indicate the possibility of the involvement of the R enantiomer of TA in the observed intestinal toxicity. Therefore, in the assessment of overall NSAID-induced GI damage contribution of both formulation and enantiomeric composition should be evaluated.

Appendix 1:

Assuming that:

1. Since the GI tract has a large surface area and only a small fraction of administered dose accumulates at the site of toxicity, the concentration of drug in the effect compartment does not have discrenible effect on the concentration of the drug in the central compartment (return from the effect to central compartment is negligible).
2. K_{1e} is 1000 fold smaller than K_{eo} (Fig. 3.21B),

Equation 1 was formulated:

$$\frac{dX_E}{dt} = k_0 + k_{1e} X_c - k_{eo} X_e \tag{1}$$

where K_{1e} and K_{eo} are the first-order rate constants for the transfer of drug from the central compartment to the effect compartment and for drug loss from the effect compartment, respectively. K_0 is zero-order input rate into the effect compartment (Fig.3.21B).

Laplace transform of equation 1 and solving for the amount of drug in the GI tract and central compartment will give:

$$\overline{X}_E = \frac{k_0 (S + k_a)(S + k_{10}) + k_{1e} k_a F \cdot D}{S(S + k_{eo})(S + k_{10})(S + k_a)} \tag{2}$$

where K_a is the first-order absorption rate constant and K_{10} is the first-order elimination rate constant from the central compartment. D and F are dose and fraction of dose absorbed, respectively.

Taking anti-Laplace of equation 2 using partial fraction theorem will yield:

$$X_E = \frac{k_0 k_a k_{10} + k_{1e} k_a F \cdot D}{k_{eo} k_{10} k_a} - \frac{k_{1e} k_a F \cdot D}{k_a (k_{10} - k_a)(k_{eo} - k_a)} e^{-k_a t} - \frac{k_{1e} k_a F \cdot D}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})} e^{-k_{10} t}$$

$$- \frac{k_0 (k_a - k_{eo})(k_{10} - k_{eo}) + k_{1e} k_a F \cdot D}{k_{eo}(k_a - k_{eo})(k_{10} - k_{eo})} e^{-k_{eo} t} \qquad (3)$$

Where X_E is the amount of drug in the effect compartment.

Rearranging and simplifying equation 3,

$$X_E = k_{1e} k_a F . D \cdot [\frac{k_0 k_{10} + k_{1e} F . D}{k_{eo} k_{10} k_a k_{1e} F . D} - \frac{1}{k_a (k_{10} - k_a)(k_{eo} - k_a)} e^{-k_a t}$$

$$- \frac{1}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})} e^{-k_{10} t} - \frac{k_o (k_a - k_{eo})(k_{10} - k_{eo}) + k_{1e} k_a F . D}{k_{eo}(k_a - k_{eo})(k_{10} - k_{eo}) k_{1e} k_a F . D} e^{-k_{eo} t}] \qquad (4)$$

Considering assumption 2 ($k_{1e} = k_{eo}/1000$), one can further simplify equation 4 to:

$$X_E = k_{1e} k_a F . D \cdot [\frac{1000 k_0 k_{10} + keoF . D}{k_{eo} k_{10} k_a k_{1e} F . D} - \frac{1}{k_a (k_{10} - k_a)(k_{eo} - k_a)} e^{-k_a t}$$

$$- \frac{1}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})} e^{-k_{10} t} - \frac{1000 k_o (k_a - k_{eo})(k_{10} - k_{eo}) + k_{eo} k_a F . D}{k_{eo}^2 (k_a - k_{eo})(k_{10} - k_{eo}) k_a F . D} e^{-k_{eo} t}] \qquad (5)$$

Setting:

$$M = \frac{1000 \cdot k_0 k_{10} + k_{eo} F \cdot D}{k_{eo}^2 k_{10} k_a F \cdot D}$$

$$N = \frac{1}{k_a(k_{10} - k_a)(k_{eo} - k_a)}$$

$$L = \frac{1}{k_{10}(k_a - k_{10})(k_{eo} - k_{10})}$$

$$Q = \frac{1000 \cdot k_0(k_a - k_{eo})(k_{10} - k_{eo}) + k_a k_{eo} F \cdot D}{k_{eo}^2(k_a - k_{eo})(k_{10} - k_{eo})k_a F \cdot D}$$

The concentration-time profile of drug in the effect compartment can be described by equation 6.

$$C_E = \frac{k_{1e} k_a F \cdot D}{V_E}[M - Ne^{-k_a t} - Le^{-k_{10} t} - Qe^{-k_{eo} t}] \tag{6}$$

where C_E is the concentration of the drug in the effect compartment.

Following equilibrium between the central and effect compartments, the net transfer of drug in and out of both compartments is equal to:

$$k_{1e} \cdot X_c = k_{eo} \cdot X_E \tag{7}$$

$$k_{1e} \cdot V_d \cdot C = k_{eo} \cdot V_E \cdot C_E \tag{8}$$

where V_d and V_E are the volumes of the central and effect compartments, respectively, and. C is the concentration of drug and.

$$V_E = \frac{k_{1e} \cdot V_d C}{k_{eo} C_E} \tag{9}$$

the equilibrium partition coefficient is simply, $K_p = C_E/C$. Substituting this in equation 9 results in:

$$V_E = \frac{k_{1e} \cdot V_d}{k_{eo} k_p} \tag{10}$$

Substituting equation 10 in equation 6 yields:.

$$C_E = \frac{k_{eo} k_a k_p F \cdot D}{V_d} [M - Ne^{-k_a t} - Le^{-k_{10} t} - Qe^{-k_{eo} t}] \tag{11}$$

The relationship between TA concentration in the effect compartment and the intestinal toxicity can best be described using the sigmoid Emax model:

$$E = \frac{E_{max} \cdot C^{\gamma}}{EC_{50} + C^{\gamma}} \tag{12}$$

where E, E_{max}, EC_{50}, and γ are the observed effect, the observed maximum effect, the concentration corresponding to 50 % of the observed maximum effect, and a number influencing the slope of the concentration-effect curve, respectively.

Again, equilibrium $C = C_E/K_p$, hence, substituting this in equation 12, yield:

$$E = \frac{E_{max} \cdot (C_E^{\gamma} / k_p)}{EC_{50}^{\gamma} + (C_E^{\gamma} / k_p)} \tag{13}$$

Substituting equation 11 in equation 13 yields:

$$E = \frac{E_{\max} \cdot [\frac{k_{eo} k_a F \cdot D}{V_d} (M - Ne^{-k_a t} - Le^{-k_{10} t} - Qe^{-k_{eo} t})]^{\gamma}}{EC_{50}^{\gamma} + [\frac{k_{eo} k_a F \cdot D}{V_d} (M - Ne^{-k_a t} - Le^{-k_{10} t} - Qe^{-k_{eo} t})]^{\gamma}} \qquad (14)$$

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